THE AMERICAN JOURNAL OF PATHOLOGY

Official Publication of
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VOLUME XVIII

1942

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PRINTED AT THE ANN ARBOR PRESS
ANN ARBOR, MICHIGAN, U. S. A.

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ORVILLE T. BAILEY, M.D., and RICHARD FORD, M.D.

(From the Departments of Pathology of the Harvard Medical School and of the Peter Bent Brigham Hospital, and from the Fourth Surgical Service of the Boston City Hospital, Boston, Mass.)

A comparatively small but pathologically interesting and clinically important group of intracranial tumors are of blood-vessel origin. Blood vessels are structures common to the tissue patterns of the brain and of organs outside the central nervous system. For this reason, tumors of blood-vessel origin in the brain and its covering might be expected to resemble in most respects those arising in the blood vessels of other organs. However, the fat, pigment and neuroglia content of intracranial hemangiomas have suggested that there are important differences between the two groups. This discrepancy has led to speculation as to the possibility of variations in the cells of origin; comparatively little attention has been paid to the histological sequences which take place during the growth of hemangiomas, regardless of the site at which they originate.

This paper presents a study of the progressive tissue changes in hemangiomas of the central nervous system and a comparison of these tissue changes with those which take place in hemangiomas of the skin and other organs. The difficulties in identifying the histological sequences concerned in the growth of intracranial tumors of blood-vessel origin are increased by their comparative rarity and the consequent difficulty in securing specimens which represent successive stages of the lesions. Furthermore, material secured at operation preserves few of the relationships of the tumors to surrounding structures. It is much easier to secure a satisfactory series of cutaneous hemangiomas. Our studies, then, have been made on three sorts of hemangiomas: a series of peripheral tumors, mostly of the skin; a group of hemangioblastomas of the brain; a group of angioblastic meningiomas. Study

* Presented at the Forty-First Annual Meeting of the American Association of Pathologists and Bacteriologists, New York City, April 11, 1941.

Received for publication, June 2, 1941.

of these three has shown that, in each, histological sequences take place which are identical when account is taken of the differences in the surrounding tissues.

Sclerosing Hemangiomas of the Skin and Other Extracranial Structures

In certain hemangiomas of the skin and occasionally in those of other extracranial organs, a process of sclerosis takes place. In connection with this, progressive tissue changes occur which result in so marked an alteration in the morphology of the hemangiomas that their true nature is often overlooked.

Wolbach,[1] in 1913, published a brief discussion of a process of sclerosis in cutaneous hemangiomas. This note described clearly the tissue sequences involved and their consequences as illustrated by two examples. In the 28 years since that publication appeared, he has collected a large series of sclerosing hemangiomas which confirms and extends the original description. A detailed report on this material has recently been prepared in his laboratory.[2] Since the alterations which occur during the process of sclerosis in cutaneous hemangiomas are directly comparable to the progressive tissue changes in tumors of blood vessels arising in the brain and its coverings, a brief review will be given here.

Cutaneous hemangiomas consist of blood vessels, among which there is a stroma of collagenous tissue which acts as a supporting framework for the neoplasm. When sclerosis occurs, the stroma increases in amount, either diffusely or in foci. It has so far been impossible to detect what starts the process of sclerosis but, once started, the process leads to a clearly defined sequence of cellular changes. The increase in connective tissue results in the occlusion of vessels, either directly or through later contraction. The red blood cells thus trapped disintegrate and their degeneration products are taken up by phagocytes. In this way, the hemangiomas come to contain hemosiderin-filled cells, at times in sufficient number to give a brown color to the tissue. The phagocytic cell nuclei are identical with those of the endothelium in the blood vessels of the tumors. Occasionally some of the pigment is taken up by endothelial cells while still in their usual location about the vascular lumina. For these reasons, it appears that the phagocytes are of endothelial-cell origin.

Some endothelial cells of the tumor take up fat—evidently fat of normal character in the circulating blood (that is, nutritional fat). It is possible that some of the fat is derived from the disintegrating red blood cells. However, the tumors in which the fat is most abun-

dant seldom contain large quantities of hemosiderin and those brown with hemosiderin usually contain small amounts of fat. The lipoid content of the hemangiomas richest in this material seems too great to be accounted for by the number of red blood cells which might have disintegrated. Therefore, it is likely that most, if not all, of the fat is derived from the blood serum. As vessels become obliterated, the relation of the fat-filled cells to their walls is obscured. In this way, large masses of cells filled with hemosiderin and lipoid material accumulate without apparent connection with the vascular lumina. The groups of fat cells give a yellow color to the parts of the tumors in which they are numerous.

In most of the sclerosing hemangiomas, giant cells make their appearance. The morphology of the nuclei and their relationship to other cells in the surrounding tissue suggest that the giant cells are of endothelial origin. In some specimens, the giant cells are few but in occasional examples they are so numerous as to be very conspicuous in the general histological picture.

The degree of sclerosis varies from specimen to specimen. It may be absent, slight, or marked. The other tissue changes will then vary accordingly. Furthermore, the proportion of fat, hemosiderin and of giant cell formation may vary greatly in different examples. If only a few specimens are studied, especially when they happen to represent different extremes of the process, a common origin may be overlooked. For this reason, some sclerosing hemangiomas have been called giant cell xanthomas, and some histiocytomas. Many other names have also been applied to tumors of this group; for instance, it appears that the giant cell endothelioma of tendon sheaths is also a sclerosing hemangioma. These tumors have been regarded, from time to time, as localized manifestations of a general disturbance in lipoid metabolism. Localized subcutaneous deposits are found in patients with such disturbances of lipoid metabolism as Schüller-Christian's disease. Careful histological study makes it certain that sclerosing hemangiomas are lesions of entirely different character than such localized lipoidal deposits. Complete postmortem examination in some of the patients with sclerosing hemangiomas has failed to show any evidence of a generalized disturbance of lipoid metabolism

General Considerations in Regard to Blood-Vessel Tumors of the Brain and Meninges

The recognition of the histological sequences just discussed has made it possible to include among the peripheral hemangiomas a considerable number of tumors previously regarded as different in histogenesis.

Because of this, it seemed worth while to examine a series of intracranial tumors originating in blood vessels to determine whether similar sequences might explain the supposed differences between these tumors and hemangiomas elsewhere.

Cushing and Percival Bailey,[3] in their monograph dealing with blood-vessel tumors of the brain, divided these lesions into angiomatous malformations and true neoplasms, *i.e.*, hemangioblastomas. A group of tumors originating in the meninges was described by Percival Bailey, Cushing and Eisenhardt [4] under the name *angioblastic meningioma.* The angiomatous malformations have not been considered in this study since they lack the characteristics of progressive tissue growth. There was available for study a series of 20 intracranial tumors of blood-vessel origin, partly from the service of Dr. Harvey Cushing and partly from that of Dr. Elliott C. Cutler. Of these, 16 were hemangioblastomas, 15 located in the cerebellum and 1 in the spinal cord. Four were of the type called angioblastic meningiomas. All tumors in which the material was fragmentary or poorly fixed were excluded from the series. Fixation was in Zenker's fluid and in a 4 per cent solution of formaldehyde. The following histological methods were used: hematoxylin and eosin, eosin and methylene blue, Mallory's phosphotungstic acid hematoxylin, Mallory's aniline blue connective tissue stain, Foot-Bielschowsky's method for reticulum, Laidlaw's method for reticulum and Mallory's method for iron. When unembedded formaldehyde-fixed material was available, Scharlach R and osmic acid were used for the demonstration of fat.

It will be noted that the terms *hemangioma* and *hemangioblastoma* have both been used in this paper. *Hemangioma* is the one most often employed by general pathologists for blood-vessel tumors of the skin and other viscera. *Hemangioblastoma* is used especially by neuropathologists to apply to blood-vessel tumors of the brain, largely as the result of the publications of Dr. Cushing's clinic. The latter term has the slight advantage of emphasizing the fact that the lesions are neoplasms and not malformations. The two terms are used interchangeably in this paper, and no special significance is intended when one is chosen rather than the other.

Hemangioblastomas of the Brain

The clinical and gross anatomical features of hemangioblastomas of the brain are well known. They are summarized in the monograph of Cushing and Percival Bailey.[3] As this publication contains an extensive review of the literature to 1928, the survey has not been repeated here. Since 1928 there have been a number of reports dealing with

the subject from various points of view but not adding material relevant to the present discussion. A useful and well illustrated paper of the group is that of Sargent and Greenfield.[5]

These tumors are much more common in the cerebellum than in other parts of the brain. Fifteen tumors in the present series were located in the cerebellum; the sixteenth was a histologically similar lesion of the spinal cord. The question of cerebral hemangioblastomas is discussed in the part of this paper devoted to angioblastic meningiomas.

Those hemangioblastomas situated within the brain substance are occasionally solid but more often form a mural nodule of a cyst. Histologically, some of them are composed of large cavernous blood spaces and others are made up of vessels of capillary size (Fig. 1). In both instances, collagenous connective tissue extends among the blood vessels so as to form a supporting stroma. The resemblance of such blood-vessel tumors to those of the skin, liver and other extracranial organs is obvious.

There are, however, other hemangioblastomas in which the vessels are separated from one another by cellular areas (Fig. 2). The cells in these regions are supported by a delicate reticulum (Fig. 3). In accordance with the view of Mallory and Parker,[6] supported by the experimental work of Wolbach,[7] we have regarded the reticulum as a stroma of fine collagen fibers. As shown in Figure 3, the silver impregnation renders more conspicuous the occasional capillaries in the cellular areas. The methods for reticulum bring out very clearly the connective tissue fibers beneath the endothelial cells. In sections prepared with other stains, the capillaries may be indistinct because they are empty and collapsed. Some of the cells in the intervascular tissue contain fat and are spoken of as "xanthoma" or "pseudoxanthoma" cells. These features and a variable number of neuroglia cells in some portions of the tumors have led neuropathologists to set these hemangioblastomas apart from other tumors of blood-vessel origin. Phagocytes containing hemosiderin, which occur in these hemangioblastomas, have attracted less attention. On the other hand, these features, except for the neuroglia, are exactly those which make their appearance as hemangiomas outside the brain undergo sclerosis.

Presentation of a representative instance of cystic hemangioblastoma of the cerebellum will assist in the discussion of the progressive tissue changes in these tumors.

Case 1

Headache, visual disturbances, personality changes. Cerebellar exploration disclosing cyst with mural nodule. Fixation of wall without removal of nodule. Gradual recurrence of symptoms after 11 years. Re-exploration 13 years after first

operation with complete extirpation of hemangioblastoma (sclerosing hemangioma) of cerebellum. Death from postoperative hemorrhage.

Mr. C. D. was a laborer, 39 years old, who was admitted to the hospital because of headaches. The onset of the patient's illness 5 years previously was characterized by attacks lasting about 1 minute in which "things would go blank," which occurred as he was getting up or lying down. After 3 months, these attacks were replaced by headaches, nausea and vomiting. The headaches increased in frequency and severity and later were accompanied by dizziness and tinnitus. The patient felt that he was becoming irritable and more easily upset than previously. Disturbances in vision made their appearance 2 months before admission here. He had been admitted to another hospital 1 month before coming to this hospital, after a period of unconsciousness lasting 12 hours. There a ventricular puncture was performed, after which the patient left against advice.

At the time of admission to the Peter Bent Brigham Hospital, the general physical examination revealed no abnormal findings. On neurological examination, there was bilateral papilledema (right eye, 3 diopters; left eye, 4 diopters) and slight nystagmus to the right. During a severe headache the Romberg sign, previously negative, became positive, the patient falling to the right. Coincident with this, marked ataxia appeared in both upper extremities and the patient leaned to the right when he walked. The nystagmus was unchanged during the attack of headache. Roentgenograms showed no changes to assist in localization of the tumor.

A cerebellar exploration was carried out. When the cerebellum was exposed, the thin wall of a cyst was brought into view. The cyst originated in the right cerebellar hemisphere near the midline. About 15 cc. of straw-colored fluid were obtained when the cyst was needled. The surface of the cerebellum was highly vascular. An incision was made in the cyst wall. After thorough fixation of the cyst wall with Zenker's fluid, the lining puckered everywhere except at a point on the lower surface of the cyst where a reddish nodule of tumor came into view. It did not seem wise to prolong the operation sufficiently to remove the nodule.

Postoperative recovery was rapid, and the patient remained symptom-free for 11 years. He then had one severe attack of headache. The only residuum of this attack was a tendency to stagger to the right. Two years later, headache again appeared at intervals and at this time was accompanied by nausea. The patient was readmitted to the hospital after 10 days of severe occipital headaches. Examination at that time elicited tenderness in the suboccipital region; the neck was somewhat rigid. Partial anosmia had made its appearance. The visual acuity was 20/200 in both eyes. The right optic disk showed slight atrophic changes and papilledema of 1 diopter. There was papilledema of 2 diopters on the left. Nystagmus to the right was present on two occasions. The patient had a tendency to stagger to the right, and fell in the same direction when the Romberg test was tried. The pharyngeal reflex was absent. Adiadokokinesia was brought out by the usual tests. The abdominal reflexes were absent on the right; all deep reflexes were hypo-active. The only abnormality in the roentgenograms was the operative defect in the skull.

Suboccipital exploration was undertaken and the field of the former procedure again exposed. After removal of the spine and lamella of the atlas, a tumor the size of a golf ball was brought into view in the right cerebellar hemisphere. The tumor was partly cystic, the cavity containing yellow fluid which immediately clotted. The entire tumor was enucleated.

The postoperative course was stormy. Because of slow pulse and respirations, the bone flap was re-elevated on the night after operation. The patient remained in an unsatisfactory condition and death occurred on the sixth hospital day.

Autopsy was limited to examination of the brain. There was a

large blood clot in the cavity from which the tumor was removed and over the cisterna magna. No residual tumor could be found.

The material removed at operation was promptly fixed and proved excellent for study. It consisted of a nodule of tumor weighing 12 gm. and measuring 3.2 by 1.8 by 2 cm. The specimen was soft in consistency. The surface was in part covered by a thin capsule while elsewhere it was irregular. Some cerebellar tissue was attached to one border. The cut surface showed pinkish gray tissue with many small yellow areas.

Microscopical examination showed the tumor to be of vascular origin. It was composed of blood vessels ranging in size from very large irregular blood spaces to minute capillaries. The larger blood vessels contained moderate numbers of blood cells. They were lined by endothelial cells, usually in one layer, though here and there small clusters of these cells could be found. The endothelium of a very few vessels was surrounded by layers of smooth muscle. In nearly all vessels, however, only connective tissue lay outside the endothelium. This varied in amount from a few collagen fibers to wide collars of partially hyalinized collagenous tissue. The capillaries sometimes contained blood but were more often empty. Their walls consisted of a layer of endothelium with collagen fibers beneath, often only a single fiber outlining the endothelial-lined space.

The intervascular tissue consisted of closely-packed cells in a delicate reticular stroma. Coarse collagen fibers were found only in occasional areas. The tumor cells between blood vessels had oval nuclei with a distinct nuclear membrane and well dispersed chromatin; mitotic figures were rare. The cytoplasm was acidophilic. The general morphological characteristics of the tumor cells in the intervascular tissue were identical with those of the endothelium lining the blood vessels. In some areas (corresponding to the regions which appeared yellow grossly), the cytoplasm of the tumor cells contained medium-sized droplets of fat, which stained orange-red with Scharlach R and black with osmic acid. Cells containing lipoid were confined almost entirely to the intervascular tissues but endothelial cells in the lining of the vessels contained fat droplets in rare instances. There were phagocytic cells filled with hemosiderin scattered in foci through the tumor. These cells were clustered together in the areas where collagen was most abundant.

In portions of cerebellum attached to the specimen, the astrocytic feltwork was increased in amount and in density. Astrocytes were included in the margin of the tumor and a few of them extended for a distance into it. The neuroglia content of this tumor, however, was not so great as that of some other neoplasms of the series.

PROGRESSIVE TISSUE CHANGES IN HEMANGIOBLASTOMAS OF THE BRAIN

It is clear that such tumors as that found in case 1 are of blood-vessel origin. The evidence for this lies not only in the number of atypical larger vessels in the neoplasm but especially in the presence of minute, poorly formed capillaries even in the most cellular portions and in the resemblance of the cells in the intervascular portions to endothelial cells. In order to explain the differences between the histology of these tumors and that of the more familiar tumors of blood-vessel origin (Fig. 1) it is necessary to find either peculiarities of the cells from which neoplasms originate or progressive sequences of tissue behavior by means of which the morphology becomes changed.

There are two important indications that histological sequences are involved rather than differences in the cells of origin. The hemangioblastomas of the cerebellum may be arranged in a series, placing at one extreme the tumors composed of fairly typical blood vessels with a collagenous stroma and at the other, tumors such as that described in case 1. Between the two extremes, a variety of intermediate stages are found. The cellular areas with fat-laden cells, the neuroglia content and the hemosiderin pigmentation may be absent, slight or marked. The likelihood of these intermediate stages being due to progressive tissue changes is enhanced by a second fact—that similar accumulations of phagocytes filled with hemosiderin and lipoid material are known to result from a process of sclerosis in hemangiomas occuring in organs other than the brain.

The sequences of sclerosis are very similar wherever the blood-vessel tumor takes origin. An overgrowth of stroma results in occlusion of some of the blood vessels. Disintegration products of blood, thus trapped, appear in phagocytes. Giant cells are occasionally found in the sclerosing hemangioblastomas of the brain, but they did not form a conspicuous part of the histological picture in any of the tumors in this series.

As already pointed out, the presence of phagocytes containing lipoid has been regarded as an important feature of the histology of hemangioblastomas of the brain. A few of the endothelial cells of still patent capillaries pick up fat, at first in small droplets and later in amounts sufficient to occupy most of the cytoplasm. A large number of the lipoid-laden phagocytes lie between the vessels. The phagocytes appear to be endothelial in origin. The evidence points to the blood stream as the source of the fat because of its presence in occasional endothelial cells lining patent vessels and because there is no indication of a generalized disturbance of lipoid metabolism. It is difficult on purely morphological grounds to exclude the possibility that some of the fat is derived from degenerating myelin. Since the sequences in which the

lipoid phagocytes appear and their distribution are similar to those of morphologically identical cells in sclerosing hemangiomas outside the central nervous system, it would appear that the fat has a common origin in all locations. This makes it unlikely that any important part of the lipoid is derived from the degeneration products of myelin.

The presence of neuroglia seems at first difficult to explain on the basis of progressive tissue changes. This can be done, however, when the nature of the stroma is considered further. The stroma of peripheral hemangiomas is derived from the connective tissue about the lesion. It consists of collagen fibers and the cells which produce and maintain them. When the stroma increases in the process of sclerosis, the change is in quantity rather than in character of the stromal cells and their fibers. In the brain, the supporting tissue under normal conditions is of two kinds. One of these is collagenous connective tissue which is associated in distribution with the blood vessels of the brain. The other is the ectodermal stroma, that is, the network of astrocytes, which participates in the support of the nerve cells and their processes. Both mesodermal and ectodermal stroma are available to take part in the histological sequences of sclerosis when they occur in blood-vessel tumors of the central nervous system.

Most of the stroma of some hemangioblastomas of the brain is composed of collagenous connective tissue. When the neoplastic blood vessels infiltrate the surrounding brain substance, the nerve cells and their fibers are destroyed as far as infiltration extends. Some of the glial supporting tissue escapes, and, being a tissue with considerable capacity for growth, its cells proliferate. They then become incorporated in the hemangioblastoma as a part of its stroma. Since the astrocytes are usually confined to the outer zone of the tumors and are in all our cases most abundant there, the indications are that the neuroglia becomes available only when the tumor has extended into the adjacent brain.

Because there are several factors in the process of sclerosis which vary independently, the number of possible histological pictures in sclerosing hemangiomas of the brain is very large. Many superficially different histological pictures may result from what is essentially one process. It is possible to overlook the fundamental similarity of specimens representing different extremes unless intermediate specimens have been studied and the progressive tissue changes defined.

So-Called Angioblastic Meningiomas

In 1928, Percival Bailey, Cushing and Eisenhardt [4] described a group of meningeal tumors of blood-vessel origin which they called *angioblastic meningiomas*. In defining the term, they stated: "By the

term *angioblastic meningioma* we do not mean meningiomas that merely happen to be highly vascular in the sense that the term angioglioma is sometimes used for a highly vascular glioma. What we do mean is that a meningeal tumor which possesses the unmistakable naked-eye appearance of the usual and familiar type of one of these lesions may prove histologically to have the same architectural features and reticular network that is possessed by the hemangioblastomas which originate within the brain itself, more frequently, if not exclusively, in the cerebellum." In other words, the distinction between angioblastic meningiomas and hemangioblastomas is one of location only.

The study of the so-called angioblastic meningiomas in the present series has confirmed the statement that these tumors are of blood-vessel origin. It has indicated, furthermore, that the same progressive tissue changes are present in these tumors as in hemangioblastomas of the brain substance, skin and other organs. It would hardly be worth while to discuss separately in this paper two groups of tumors differing only in location if it were not for the fact that a number of publications have appeared in which either the similarity of hemangioblastomas of the brain and angioblastic meningiomas has been overlooked or an attempt has been made to relate angioblastic meningiomas to the meningeal tumors originating in arachnoid cells. The literature dealing with angioblastic meningiomas has recently been summarized by Courville and Abbott.[8]

The lesions of this type described in publications have been located almost entirely above the tentorium. However, some few cerebellar tumors can hardly be excluded from the group. Two of the tumors in the present series were located in the occipital lobe, the other two in the cerebellum. The cerebral tumors presented the characteristics of angioblastic meningiomas as described by Percival Bailey, Cushing and Eisenhardt [4] and elsewhere. We have chosen, however, for detailed report one of the cerebellar tumors with several atypical features. It arose from the meninges and pushed the cerebellum before it, infiltrating its structure only very slightly. Thus, although it behaved in respect to the brain tissue as typical angioblastic meningiomas do, it had no dural attachment. Since it had not been approached surgically, the relations to brain and meninges could be studied. The tumor has many features both of hemangioblastoma and of angioblastic meningioma. It may be regarded as a transitional form between the two categories, but it seems to us to strengthen the opinion that hemangioblastomas of the brain and angioblastic meningiomas are, from the pathological standpoint, one and the same, except in point of origin.

Case 2

Headaches, vomiting, visual disturbances and personality changes. Subtemporal decompression performed elsewhere. By ventriculography, poor filling of third ventricle with displacement of its posterior portion to the left. Death from bronchopneumonia. Sclerosing hemangioma of cerebellar meninges found at autopsy.

Mrs. M. LeB. was a French-Canadian mill-worker, 53 years old, who came to the hospital because of headache and visual difficulties. For the year preceding admission, she experienced headaches, which became very severe during the last 5 months of the interval. As the headaches increased in severity, they came to be associated with vomiting, not preceded by nausea. About 6 weeks before entering the hospital, she noticed blurring of vision in the right eye, followed by blindness in that eye 9 weeks later. Four weeks before entry, vision became blurred in the left eye. The patient's memory began to fail; there were visual and auditory hallucinations on several occasions. Two weeks before admission here, a subtemporal decompression had been done elsewhere with partial relief of symptoms. The patient was transferred to the Peter Bent Brigham Hospital for further study and treatment.

On arrival at this hospital, the only abnormalities noted on general physical examination were bulging in the area of subtemporal decompression and moderate cardiac enlargement. The neurological examination revealed the sense of smell to be somewhat diminished. There was no perception of light in the right eye. The right pupil did not react to accommodation or to light directly, though there was a reaction to light consensually. The palpebral fissure was narrowed and internal strabismus of the right eye was present. There was marked diminution of vision in the left eye and the pupil was dilated but not fixed. Papilledema was present to the extent of 3 diopters in the right eye and 1 diopter in the left eye. There was slight nystagmus to the right. Both corneal reflexes were sluggish and increased lacrimation was noted in both eyes. There was a partial left lower facial palsy. A definite dysarthria was combined with a tendency to loquaciousness. The patient responded to tests for adiadokokinesia slowly but accurately. Temperature sensation was absent over the entire left half of the body from crown to toe, as were tactile sensation and ability to discriminate pain and pressure in the same area. The pharyngeal reflex was absent. Tendon reflexes were active and equal; the right upper was the only abdominal reflex obtained. Stereoroentgenograms of the skull showed no definite localizing signs of intracranial tumor.

Ventriculography was carried out on the fourth hospital day. The ventriculograms showed definite dilatation of both lateral ventricles but no localized deformity. The third ventricle filled poorly; its posterior portion was displaced slightly to the left. The patient's postoperative course was stormy and death occurred on the 13th hospital day, further operation not being carried out.

Anatomical diagnoses: Sclerosing hemangioma of meninges; bronchopneumonia; bacteremia (*Staphylococcus aureus*); multiple abscesses of lungs; arteriosclerosis, generalized; multiple leiomyomata of uterus.

The findings relevant to the present discussion were limited to the brain. There were no hemangiomas in other organs.

The region of the subtemporal decompression was bulging. The dura was tense, the convolutions flattened and the sulci narrowed. There was a tumor 1 cm. deep and 2 cm. in length in the posterior portion of the right cerebellar lobe. This was attached to the leptomeninges and bulged above the adjacent brain tissue, but there was no dural attach-

ment. The tumor had a distinct yellowish color. Not only was the tumor itself highly vascular but the blood vessels of the meninges about it were increased in size and number.

On cross section the fresh tumor was predominantly orange in color. The relations of the neoplasm were best studied in large sections, which included a cross section of the entire tumor and adjacent portions of the cerebellum (Fig. 5). This showed that the tumor was situated superficially with its outer surface covered only by an attenuated arachnoid membrane. The neoplasm occupied largely a position corresponding to a greatly widened subarachnoid space. As a result, the underlying cerebellar folia were pushed aside and flattened while adjacent cerebellar folia were normal in size and shape. The tumor, then, appeared from its gross relationships to have arisen in the leptomeninges and to have caused its effects on the cerebellum by pressure. Closer inspection, however, showed that the superficial portions of some of the folia had been invaded for a short distance. Although the tumor was primarily a tumor of the leptomeninges, it extended through the pia for a short distance into the underlying cerebellum (Fig. 7). In this it resembled the meningiomas of arachnoid-cell origin which pass the pial barrier.[9, 10]

Microscopical Examination. The tumor was characterized by the presence of blood vessels separated by areas of closely packed cells. The vascular spaces varied in size from large, irregular vessels to minute capillaries. The vessels were lined with flattened endothelial cells in a single row, or with swollen, closely set endothelial cells in small masses. Some of these cells contained granules of hemosiderin, and in others there were droplets of lipoid material in the cytoplasm. The endothelial cells were surrounded in the larger blood spaces by a rather thin zone of collagenous tissue. Occasionally, the collagen bundles were swollen and separated by edema so that there were large, relatively acellular collars around the vessels. About the endothelium of the finer capillaries, single delicate strands of collagen blended with the reticulum of the intercapillary cell clumps (Fig. 11).

The tissue separating the blood vessels was composed of closely packed cells in a delicate reticulum. At intervals, however, minute empty capillaries could be outlined with reticulum stains (Fig. 11). The cells in the intervascular tissue were endothelial in type. The oval nuclei were surrounded by a distinct nuclear membrane and the moderate amount of chromatin was well dispersed. Mitotic figures were occasionally encountered but were not numerous. The cytoplasm of the tumor cells was acidophilic and in some cells contained granules of hemosiderin (Fig. 8). These pigment phagocytes were concentrated

in areas where collagen was abundant and tumor cells relatively few. In a large percentage of the cells, there were droplets of fat in the cytoplasm (Fig. 9). Usually these fat-filled cells were packed in large masses separated by bands of collagenous tissue from which delicate reticular fibers extended into the masses and formed a stromal network for the fat-filled cells. Occasional multinucleated giant cells were found in the intervascular tissue.

The free edge of the tumor was covered by an intact layer of arachnoid cells with rather dense collagen fibers beneath it. While the fat-filled cells were abundant throughout the tumor, they were especially numerous near the free edge of the neoplasm. Neuroglia cells were not present in this region. On its deep surface, the tumor invaded for a short distance the granular and molecular layers of the underlying cerebellar cortex. In the part of the tumor nearest the region of invasion, numerous fine capillaries, intercapillary endothelial cells, collagen fibers and astrocytes were mingled in haphazard fashion (Fig. 10). The astrocytes extended into the tumor for some distance toward the center. Occasionally, small groups of astrocytes surrounded capillaries and were attached to their walls by sucker feet. Only very close to the advancing edge were there any nerve cells in the tumor. The architecture of the cerebellar cortex immediately below the neoplasm was somewhat distorted from pressure but elsewhere it was normal (Figs. 5 and 7).

A Comparison of Angioblastic Meningiomas and Hemangioblastomas of the Brain

As pointed out earlier in the discussion, case 2 has features which are in part those of the tumors described as hemangioblastomas of the cerebellum and in part those of angioblastic meningiomas. Similarities to cerebellar hemangioblastomas are its location below the tentorium and its lack of dural attachment. The resemblance to angioblastic meningiomas is its origin from the meninges. The detailed histology of both varieties of tumors is identical, as pointed out by Percival Bailey, Cushing and Eisenhardt.[4] Case 2, therefore, seems important in studying the resemblances and differences of hemangioblastomas of the brain and angioblastic meningiomas.

The angioblastic meningiomas are solid tumors of meningeal origin and are by far more frequent above the tentorium than below. It has been suggested by some writers that all cerebellar tumors of vascular origin should be excluded from this group. However, the neoplasm in case 3 of the report by Percival Bailey, Cushing and Eisenhardt [4] probably originated in the cerebellar meninges, although at autopsy the

tumor was found in the occipital region as well as in the cerebellum. This statement is made because the initial symptoms were those associated with cerebellar involvement; symptoms of occipital lobe involvement did not appear until 2 years after the first operation. This and an example in the present series (in addition to case 2) indicate that angioblastic meningiomas may arise in the cerebellar meninges.

In contrast, the hemangioblastomas are rarely seen in the cerebrum. Cushing and Percival Bailey's [3] examples of this lesion were all cerebellar. Some of the reported hemangioblastomas of the cerebrum seem identical with those of the cerebellum. The hemangioblastomas may be solid or partly cystic but more often form a mural nodule of a cyst, which is elsewhere lined by a condensation of glia. For this reason, the tumor described by Barnard and Walshe [11] as a capillary hemangioma of the cerebrum seems certainly acceptable in the group of hemangioblastomas of the brain substance. There was a cyst in the right temporal lobe with a mural nodule identical in histology with the nodules in hemangioblastomatous cysts of the cerebellum. It is interesting to contrast this lesion with the tumor in case 2 of the present series. Barnard and Walshe's tumor was a cyst with a mural hemangioblastomatous nodule in the substance of the cerebrum when statistically a solid tumor of the cerebral meninges would be expected in that location. The lesion in case 2 of the present series was a solid tumor of the cerebellar meninges when statistically a cyst with a mural nodule would have been expected. It seems more logical to say that hemangioblastomas of the cerebrum are usually solid and attached to the meninges while those of the cerebellum usually form the mural nodule of cysts rather than to set these lesions apart as separate entities.

It has been pointed out that angioblastic meningiomas have an attachment to the dura.[4, 8, 12] The absence of dural attachment in the tumor of case 2 might be regarded as evidence that the tumor is not an angioblastic meningioma. However, it should be recalled that the tumor was comparatively small and was covered by intact arachnoid. A small angioblastic meningioma of the left frontal lobe without dural attachment has been reported by Wolf and Cowen,[12] case 4. The tumors attached to the dura are larger than these two neoplasms. The situation seems best explained by assuming that all were originally confined to the leptomeninges, as was the tumor in case 2. Enlargement of the neoplasm would lead to a break in the continuity of the layer of arachnoid cells. As discussed elsewhere,[10, 13] the dura participates in the repair of the defect thus produced. Because of this, any tumor which leads to discontinuity of the superficial layer of the arachnoid

becomes attached to the dura, no matter what its cell type is. Dural attachment of angioblastic meningiomas seems to depend on the production of a defect in the outer layer of the arachnoid with participation of tissue from the dura in filling the defect.

There are no essential differences in the histology of angioblastic meningiomas and hemangioblastomas of the brain. Each is composed in part of blood vessels of considerable size; in part, of more cellular tissue in which the only blood vessels are a few small capillaries. By the same sequences as those described in peripheral sclerosing hemangiomas, increased stroma, hemosiderin and fat make their appearance both in hemangioblastomas of the brain substance and in angioblastic meningiomas. Neuroglia occurs in both tumors; its source in hemangioblastomas has already been discussed.

Since neuroglia is not found in the brain envelopes, it has been suggested that the neuroglia of angioblastic meningiomas makes its appearance as the result of a congenital glial heterotopia in the meninges and that it forms, with the blood vessels, a complex congenital overgrowth of tissues. Although the angioblastic meningiomas for the most part push the underlying brain ahead of them, they extend into it by direct invasion for a short distance, with the result that portions of brain tissue come to be included within the tumor. The neurons in such areas are destroyed but the astrocytes survive and continue to grow, responding to the tumor so as to form a portion of its stroma. When further extension of the neoplasm takes place, the astrocytes persist and finally vessels of the tumor, neuroglia and collagen fibers are mixed together (Fig. 10). These histological sequences are identical with those encountered at the margins of hemangioblastomas of the brain substance.

It was definitely stated by Percival Bailey, Cushing and Eisenhardt [4] that the term *angioblastic meningioma* connotes a meningeal tumor of vascular origin and not a highly vascular neoplasm of arachnoid cells. In spite of this, various writers have included in their series tumors of both types or have regarded all the angioblastic meningiomas as vascular, cellular tumors originating in arachnoid cells (*vide* Bergstrand and Olivecrona [14]). The presence of a single psammoma body or of a few arachnoid cells in an angioblastic meningioma [12] represents the inclusion of tissue of the adjacent arachnoid by the expanding tumor. The neoplasms arising in arachnoid cells and commonly called *meningiomas*, no matter how vascular, are fundamentally different from the angioblastic meningiomas.

It is not necessary to postulate unique features of the cells from which angioblastic meningiomas arise to account for any of the histological features of the neoplasms. The histological features considered

to set this group apart from other tumors of blood-vessel origin can be explained by the progressive tissue changes of sclerosis. The study of blood-vessel tumors of the central nervous system would be greatly simplified if it were recognized that the tumors now called angioblastic meningiomas and those called hemangioblastomas of the brain substance are sclerosing hemangiomas of the central nervous system.

The histological sequences which take place in sclerosing hemangiomas of the brain and its coverings are reflected in the gross appearances of the tumors. In the portions where lipoid-filled phagocytes are abundant, the tissue has a yellow color. There is a brownish tint where hemosiderin has accumulated in large quantities. For this reason the otherwise reddish gray tumors become mottled with yellow, brown and orange areas. This gross appearance in a tumor, in and about which there are many very large blood vessels and blood-containing spaces, permits the identification of sclerosing hemangiomas of the central nervous system when they are exposed at operation. In case 1, the surgeon described the lesion at the first operation as red in color. Yellow areas were seen when the tumor was again exposed 13 years later. While it is possible that the yellow areas were overlooked at the first operation, the observation offers some confirmatory evidence that these fat deposits gradually make their appearance as the result of progressive tissue changes in hemangiomas.

The recognition of these tumors during a surgical procedure has a bearing on their management. In view of the speculation whether peripheral sclerosing hemangiomas may be an indication of a systemic disturbance in lipoid metabolism, it seems worth while to state again that the accumulation of fat in these tumors, central or peripheral, is not the result of a generalized disorder but of local tissue reactions. The sclerosing hemangiomas both of the meninges and of the brain substance extend into the adjacent brain instead of pushing the nervous tissue away, as do the meningiomas of arachnoid cell origin. For this reason, some of the grossly normal-appearing brain tissue about the sclerosing hemangiomas should be removed along with the tumors if complete extirpation is to be accomplished.

The progressive sequences of tissue behavior involved in sclerosing hemangiomas wherever found are in general to be regarded as regressive in character. On the other hand, the vessels of the neoplasms may continue to grow and especially the endothelial cells between the vessels may proliferate without the formation of vascular channels. Mitoses are usually found in these tumors and are abundant in some of the specimens. For this reason, sclerosis in hemangiomas is not in itself assurance of complete quiescence of the tumor cells. Enlargement

of the lesions may be due either to continued growth of neoplastic cells or to the process of sclerosis itself, on account of the increase in the amount of stroma and the accumulation of fat and hemosiderin.

The progressive tissue changes in sclerosing hemangiomas of the central nervous system indicate the extent to which the morphology of tumors may be modified by secondary histological sequences. When these are taken into consideration, many of the difficulties in morphological interpretation are removed and a more accurate definition of histogenetically related groups of tumors is made possible.

Summary

The neoplasms of blood-vessel origin in the central nervous system have been divided into two groups: the hemangioblastomas, arising in the brain substance and by far most frequently in the cerebellum; and the so-called angioblastic meningiomas. The presence of phagocytes containing fat and hemosiderin as well as of neuroglia in tumors of both groups have tended to set them apart from hemangiomas outside the central nervous system.

Study of a series of hemangioblastomas of the brain substance indicates that they may undergo a process of sclerosis entirely comparable to that found in hemangiomas of the skin and other organs. The sclerosis comes about by an increase in stroma, in large part collagenous connective tissue but in part neuroglia. In other words, both mesodermal and ectodermal supporting tissue participates in the process. The occlusion of blood vessels resulting from the increase in stroma is accompanied by an accumulation of lipoid material and hemosiderin in phagocytic cells of endothelial origin. Since the sclerosis may be absent, slight or marked, many apparently different tumors may be shown to have a common origin.

The so-called angioblastic meningiomas are hemangiomas of the meninges in which a similar process of sclerosis has occurred. These tumors infiltrate the brain to some extent; in this way, neuroglia comes to participate in the progressive tissue changes occurring in the hemangiomas of meningeal origin.

There is no fundamental difference between the two groups of tumors usually called hemangioblastomas of the brain and angioblastic meningiomas. They are both sclerosing hemangiomas of the central nervous system.

The recognition of these sequences in hemangiomas of the brain and its coverings has a bearing on the operative management of the lesions.

The study of the progressive tissue changes in sclerosing hemangiomas of the central nervous system has been facilitated by observation

of similar changes in hemangiomas of the skin and other organs. This is one of the many instances in which a lesion apparently unique to the brain can be shown to have its exact counterpart in lesions of other viscera when account is taken of the differences in the normal structural patterns of the organs concerned.

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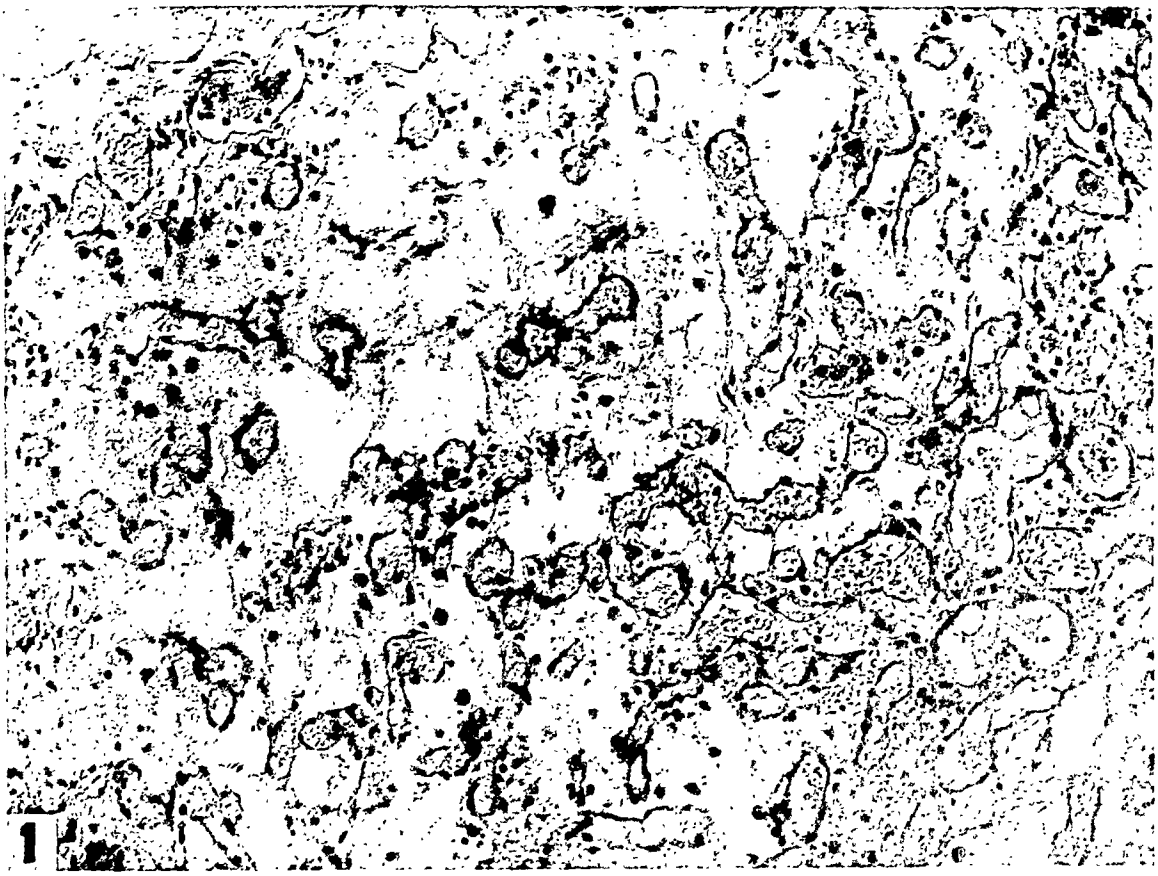
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DESCRIPTION OF PLATES

PLATE 1

FIG. 1. A hemangioblastoma of the central nervous system, without evidence of sclerosis. The tumor is composed of thin-walled capillaries separated from one another by a very delicate and edematous stroma. There are no cells containing hemosiderin or fat. Hematoxylin and eosin stain. × 150.

FIG. 2. A hemangioblastoma of the central nervous system with evidence of sclerosis. The capillaries are separated from one another by a cellular tissue. Mallory's phosphotungstic acid hematoxylin stain. × 150. (Fig. 4 shows the character of the cells of this tumor at higher magnification.)

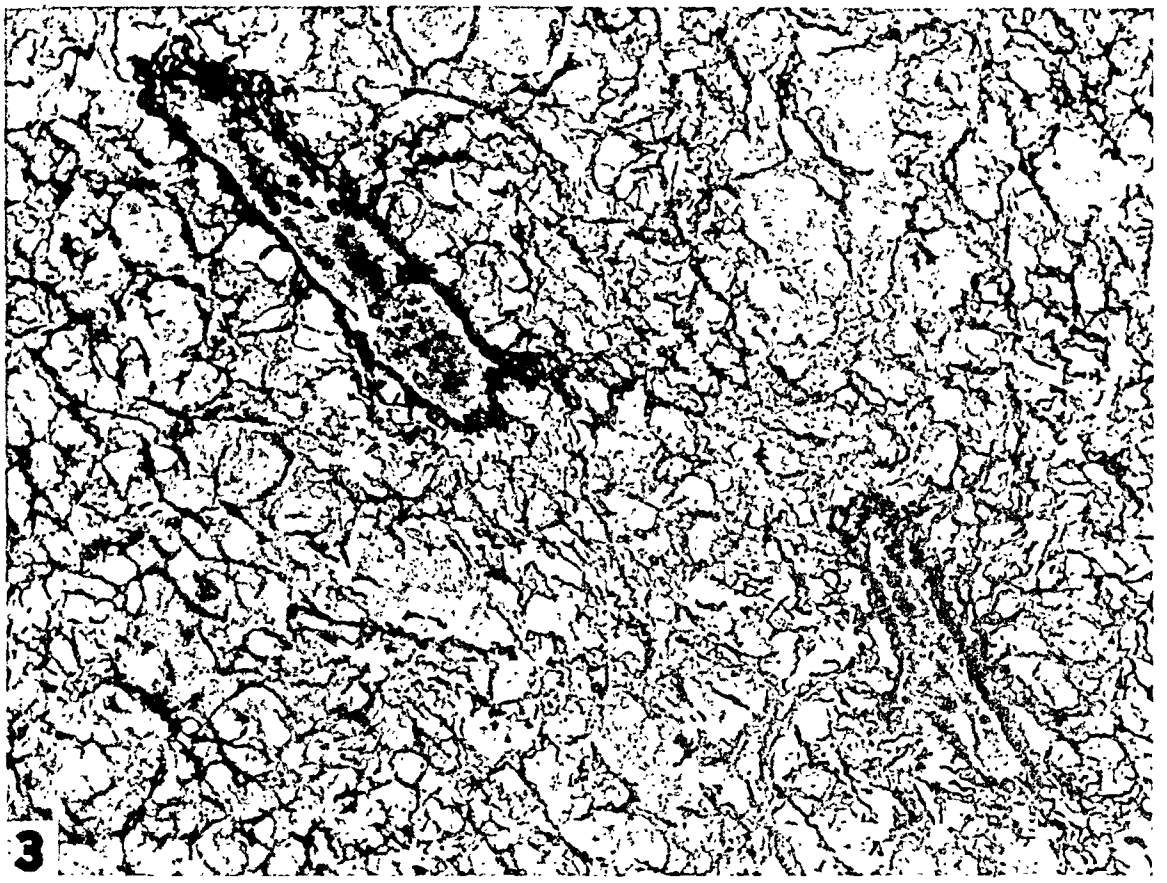

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PLATE 2

FIG. 3. The reticulum in a sclerosing hemangioblastoma of the cerebellum. Two capillaries have been clearly outlined by the stain. A delicate reticulum extends among the cells of the intervascular tissue. Laidlaw's method for reticulum. × 300.

FIG. 4. The character of the cells in the tumor shown in Figure 2. Around the thin-walled capillaries there are numerous cells with nuclei resembling those of endothelial cells. Their cytoplasm contains fine vacuoles, due to the presence of fat. Mallory's phosphotungstic acid hematoxylin stain. × 900.


3

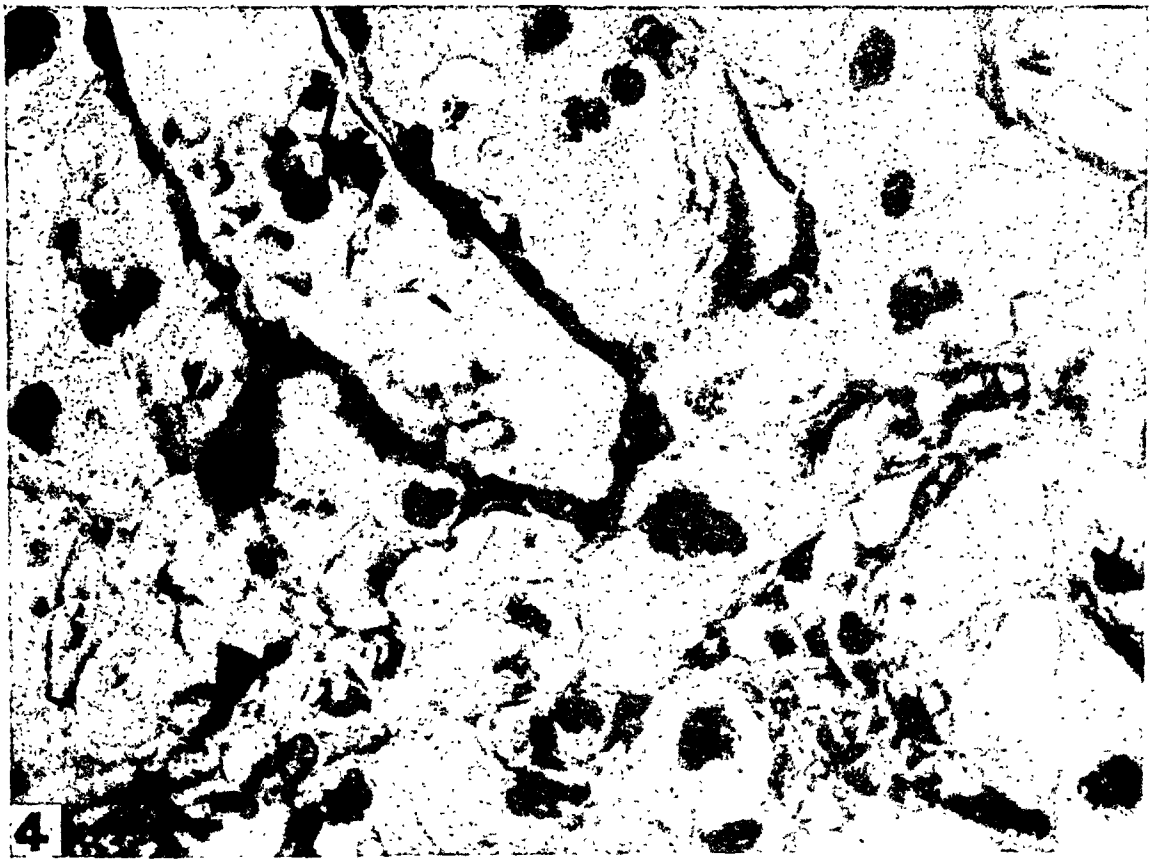

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PLATE 3

FIG. 5. The relations of the tumor in case 2 to surrounding structures. In the attachment to the meninges, the lack of cyst formation and the compression of the underlying brain, it resembles an angioblastic meningioma. The location in the cerebellum and the absence of attachment to the dura are characteristics usually associated with hemangioblastomas of the brain substance. Mallory's phosphotungstic acid hematoxylin stain. × 5.5.



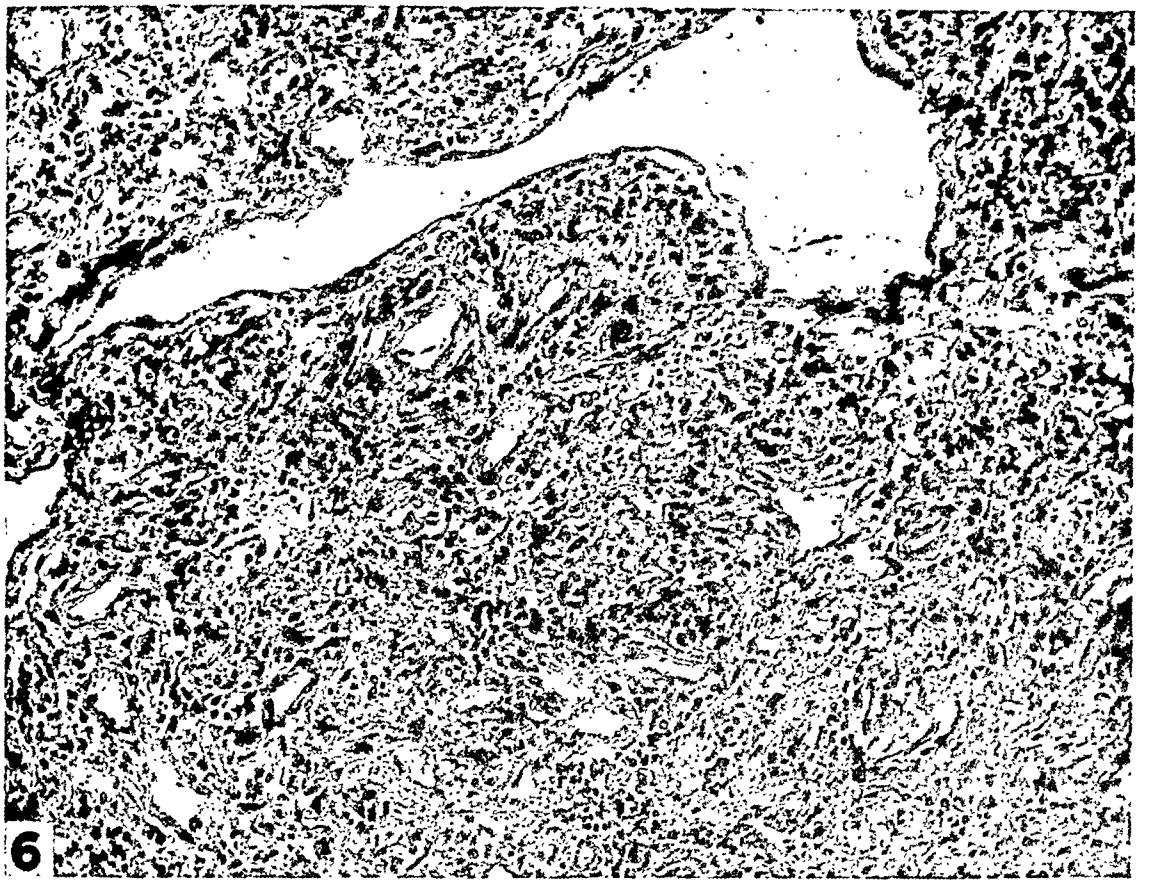
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PLATE 4

FIG. 6. The tumor in case 2 is composed of blood vessels of various sizes separated from one another by cellular tissue. The resemblance to the tissue shown in Figure 2 is striking. Mallory's phosphotungstic acid hematoxylin stain. × 150.

FIG. 7. Case 2. This shows the edge of the tumor in one region in which it infiltrates the substance of the cerebellum. Mallory's phosphotungstic acid hematoxylin stain. × 150.





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7

PLATE 5

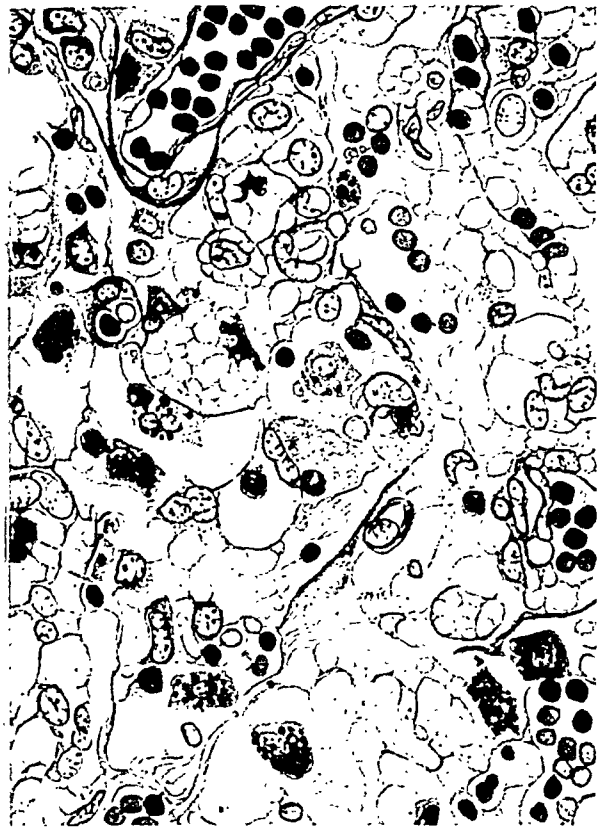
In Figures 8 to 11, the cells in the intervascular tissue are shown as they appear in sections stained by various technics.

FIG. 8. Case 2. Some of the cells in the intervascular tissue contain hemosiderin. Mallory's ammonium sulfide-potassium ferricyanide method for iron. × 697.

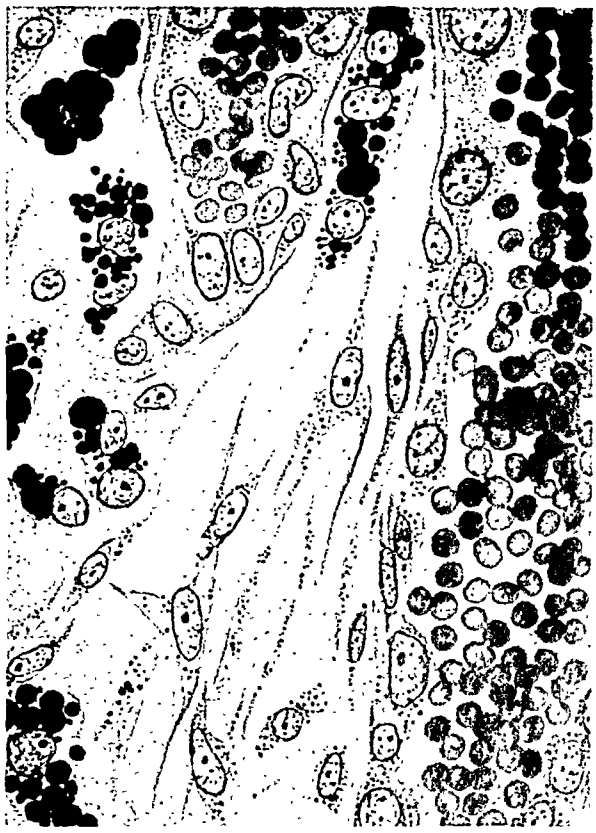
FIG. 9. Case 2. Other cells in the intervascular tissue contain fat. Scharlach R stain. × 697.

FIG. 10. Case 2. In the region in which the neoplasm has infiltrated the cerebellum, astrocytes are incorporated in the tumor. This is representative of many fields in which capillaries, collagenous tissue and astrocytes are mixed together in a haphazard manner. Mallory's phosphotungstic acid hematoxylin stain. × 697.

FIG. 11. Case 2. The reticulum outlines a small capillary in the intervascular tissue. A delicate network of these fibers extends among the phagocytic cells in the region. Foot's method for reticulum. × 697.



8



9



10



11

Bailey and Ford

Hemangiomas of the Nervous System



THE PRODUCTION OF CARDIAC AND RENAL LESIONS IN RATS BY A DIET EXTREMELY DEFICIENT IN POTASSIUM*

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In 1937, Schrader, Prickett and Salmon [1] described pathological alterations in the heart, kidneys, pancreas and intestines of rats which had been placed on a diet deficient in potassium. More recently, by restricting the dietary potassium, Thomas, Mylon and Winternitz [2] have confirmed the cardiac lesions reported by Schrader, Prickett and Salmon in rats, though they failed to note any change in the other tissues. They were also able to produce the same myocardial lesions in hogs. However, they concluded that both in the latter animals as well as in rats the development of the lesions was dependent on a diet not only deficient in potassium but in vitamin B_6 as well. Sykes and Alfredson [3] have noted electrocardiographic changes in calves on diets of low potassium content.

The present study deals with the pathological alterations in the tissues of rats placed on a diet adequate in all known respects save in its content of potassium (0.01 per cent). The composition of this diet was as follows: wheat gluten, 4.0; lactalbumin (highly purified), 10.0; gelatin, 4.0; salts, no. 21, 4.6; butter fat (purified), 8.0; dextrose to 100 per cent; viosterol, 15 drops per Kg. of diet; liver concentrate (Lederle), approximately 3.5 gm. of liver per rat per day; thiamin, 20 γ per rat per day, and vitamin E concentrate, 3 mg. per rat per day. The growth, behavior and metabolism of animals placed upon this diet are being reported elsewhere by Orent-Keiles and McCollum.[4]

MATERIALS AND METHODS

Ninety rats of the McCollum strain, about equally divided as to sex, have been examined. Their ages, when placed upon the potassium-low diet, varied from 25 to 28 days. They were killed after being on the diet from 4 to 327 days. Approximately one-half of the animals served as controls and received the potassium-supplemented basal diet either *ad libitum* or in amounts restricted to the intake of the potassium-deficient animals. The latter precaution was taken to exclude, as far as possible, any rôle inanition might have in producing the changes

* This work has been supported by a grant to the Department of Biochemistry, School of Hygiene and Public Health, from the Rockefeller Foundation.
Received for publication, April 24, 1941.

observed. Seven of the animals were born from mothers that had been placed on the potassium-low diet 10 days before their birth and had continued on the diet during suckling. They remained on the diet after weaning as long as 67 days of age.

The animals were killed with ether and autopsied immediately. Tissues were placed in Zenker-formaldehyde solution and in a 4 per cent solution of formaldehyde alone. After imbedding in paraffin, they were stained with hematoxylin and eosin. Bones were decalcified in 5 per cent nitric acid. Since it seemed inadvisable to examine all tissues from every animal, a preliminary group of animals was studied thoroughly and then those organs which showed any deviation from the normal controls were studied more minutely in other animals in order to trace the development and course of the changes. The following tissues were studied: heart, lung, liver, spleen, pancreas, adrenal, kidney, esophagus, stomach, intestine, ovary, uterus, vagina, testis, seminal vesicle, prostate, thyroid, parathyroid, thymus, arteries, skin, skeletal muscle, brain, hypophysis, bone and bone marrow. Only the positive gross and microscopical findings will be described. There were marked differences in the gonads and accessory sexual apparatus, thymus and bones of the animals fed *ad libitum* and of the potassium-deficient animals, but when the factor of inanition was ruled out by paired feeding these differences were not present.

Gross and Microscopical Changes

Heart

Grossly, changes were not seen in the heart before the third week. At 15 days, however, with the aid of binocular loupes, tiny, opaque grayish areas could be seen beneath the epicardium of some of the deficient animals. By the sixth week these lesions could be detected with the naked eye and from then on these areas were seen in practically all potassium-deficient animals. The controls showed nothing. The only other gross finding of note was a difference in the weight of the hearts of the deficient animals and their controls fed *ad libitum*. The ratio of heart weight to body weight indicated that on the whole the hearts of the former group were the larger, but interpretation of this is not accurate because we do not know how much of a rôle inanition was playing. However, some light was cast on the assumption that there was a true hypertrophy by the fact that in a group of seven animals (four deficient and three controls) on the paired feeding regimen for 87 days, the deficient animals weighed 38 per cent less than their controls, but their hearts weighed 13 per cent more than those of the latter.

Microscopically, the earliest change to be noted was a loss of striation of individual muscle fibers. This was seen either in isolated fibers or in groups of fibers after the animals had been on the potassium-low diet for 8 days. The hyaline fibers next lost their affinity for eosin and took on a grayish yellow color. After this they disappeared altogether, leaving an empty membrane. No fatty change could be demonstrated with the Scharlach R stain. Coincident with the change in the myofibrils was one which was found in the nuclei. As the fibers were undergoing necrosis the nuclei began to shrink and very soon underwent karyolysis or karyorrhexis. Associated with these changes there was an emigration of leukocytes into such areas (Fig. 1). In the early stages there were polymorphonuclear leukocytes while later mononuclear phagocytes predominated. There was a tendency for the earlier lesions to be more numerous beneath the epicardium and endocardium but necrotic fibers were found also in the center of the ventricular walls. No reaction was ever seen on the surface of the epicardium or endocardium. Lesions were scanty in the auricular musculature. Mural thrombi were not found in the ventricular chambers. Aside from the leukocytic infiltration in the zones of necrosis, no other cellular response was found. There were no perivascular accumulations of leukocytes, nor any changes in the vessels themselves.

From the eighth to the fifteenth day these lesions increased both in size and in number, so that at 2 weeks numerous large areas, covering as much as two low-power microscopical fields in greatest diameter, could be found (Fig. 2). In some animals the process took on the appearance of a diffuse myocarditis with widely scattered necrotic fibers surrounded by leukocytes. Other areas suggested the lesions that are encountered in human diphtheria. In the ensuing weeks the involvement became more extensive so that by the sixth week the necrotic foci were extremely numerous. By this time, too, there was an increasing proliferation of connective tissue at the sites where earlier lesions had been present. These scarred regions consisted of single fibers or groups of intact muscle fibers separated by connective tissue cells and bundles of collagen (Fig. 3). Scattered about, too, were mononuclear leukocytes. It was interesting to note old scarred lesions close to muscle fibers which were undergoing the necrotic changes as described. From the sixth week to the end of the period of observation in the 47th week the scarring became more extensive, while in contrast there were fewer fresh lesions. However, it must be noted that lesions indicating recent myocardial damage were found in animals on the deficient diet as long as they were observed (327 days).

When the animals which had been born to rats on the deficient diet

were examined, the same myocardial lesions were seen. At 4 weeks of age there were fresh necroses together with scars, and these same changes were found in animals examined in the sixth, eighth and tenth weeks after birth. Because of the report [5] of cardiovascular lesions in calves due to magnesium deficiency, a group of animals was given magnesium, in excess of that in the basal diet. This supplement had no effect on the development of the lesions.

In summary, in the myocardium of all animals which had been on the potassium-deficient diet 8 days or longer there occurred necrosis of the muscle fibers. Such areas then became infiltrated with leukocytes and healing took place with a proliferation of connective tissue. Similar lesions were not found in the control animals on the same diet plus an adequate potassium intake.

Kidneys

Grossly, the most prominent change in the kidneys was an increase in size of those of the potassium-deficient animals. Most marked from the sixth week on, the difference was most impressive in the paired feeding group where, although the deficient animals weighed less than their restricted controls, the kidneys of the former group weighed more than those of the latter animals. This was not only true of the wet weight but of the dry weight as well. The surfaces were finely pitted and they were paler than the corresponding control organs.

Microscopically, the first change to be noted appeared after the animals had been on the potassium-deficient diet for 8 days. In the cytoplasm of the cells of the convoluted tubules small vacuoles appeared between the basement membrane and the nucleus. When frozen sections were stained with Scharlach R, these droplets were found to be lipid in nature (Fig. 4). When examined through the polarizing microscope they were not doubly refractive. By the 14th day the lipid had increased so that most of the cells of the convoluted tubules contained it. However, by this time other changes were present. Necrotic epithelial cells as well as swollen cells with poorly staining nuclei could be found. These cells became detached from the basement membrane and lay as pink hyaline masses in the lumina of the tubules. Coagulated pink-staining material was found in other lumina. There were also dilated tubules lined by flattened epithelium, which had apparently regenerated (Fig. 5). A few multinucleated tubular epithelial cells were found. In one kidney at 15 days a few "calcified" epithelial cells were found. During this early period no changes could be detected in the glomeruli or blood vessels.

By the sixth week there were many dilated tubules lined by flattened

epithelium (Figs. 6 and 7). At this time an additional feature was added to the picture. In a few tubules there appeared a blue-staining, granular material. One or two such casts could be found in the tubules in each section and as time went on they became more numerous. A few calcified epithelial cells were noted also. The only other finding of importance was an increase in size of the individual epithelial cells when compared with the same cells from control animals on the paired feeding regime. At the twelfth week and from then on to the end of the experiment the dilatation became more marked. The amount of "calcified" material did not increase greatly or reach the degree reported in magnesium deficiency [6] and following parathormone administration.[7]

In summary, the kidneys showed necrosis of the tubular epithelium, dilatation of the tubules and later deposition of "calcified" material in the lumina of some of the tubules.

Discussion

By feeding rats a diet extremely low in potassium but adequate in all other known constituents, necrosis of the cardiac musculature and damage to the renal tubular epithelium have been produced. The other tissues appeared normal. These myocardial lesions are apparently exactly similar to those observed in rats by Schrader, Prickett and Salmon [1] and in both rats and hogs by Thomas, Mylon and Winternitz.[2] However, although the cardiac lesions are alike, there are discrepancies noted in the other organs in these three studies. Schrader, *et al.* also described alterations in the kidneys similar to those here described and, in addition, lesions in the pancreas and intestine. They failed to eliminate the factor of partial inanition. Thomas and his associates noted lesions in the heart only. The compositions of the diets used in these three studies differ. McCollum, Orent-Keiles and Day[8] have already criticized the diet employed by Schrader, Prickett and Salmon as being inadequate in other dietary essentials aside from potassium. The diets used by Thomas, Mylon and Winternitz were deficient in factors of the vitamin B complex other than thiamin, riboflavin and B_6. In addition, their diet I contained an excess of cod liver oil and was deficient in vitamin E. These workers concluded that potassium was not the only factor in producing the lesions but that a deficiency of vitamin B_6 was also necessary. These differences in the diets employed may be sufficient to explain the discrepancies in the lesions produced in these experiments.

Since the classic studies of Ringer.[9] in 1882, the importance of potassium in the normal physiology of the heart has been well recognized.

The exact mechanism for the production of cardiac lesions by diets low in potassium is quite obscure. Unfortunately, at this time, we do not know how much the blood-potassium level was affected in the rats on our potassium-low diet. The potassium content of the heart muscle, however, was 35 per cent lower than that of the controls. When one attempts to apply these experimental findings to disease in man associated with low blood potassium there is very little available information. In familial periodic paralysis there is a low blood potassium during attacks and at such time electrocardiographic changes have been described.[10] Another possibility which comes to mind is the lowering of serum potassium in patients with Addison's disease to whom an excessive amount of desoxycorticosterone acetate had been administered. Here, cardiac failure has been described,[11] though it may be due to some factor other than lowering of the serum potassium. To our knowledge no histological studies on such hearts have been reported.

It is worth while pointing out that the cardiac musculature was the only type affected. The striated myofibrils as well as the involuntary fibers were not damaged, even though the potassium content of the voluntary muscle was lower than that of the myocardium.

The changes in the kidneys are fairly characteristic of the so-called "necrotizing nephroses" which can be produced by various chemical poisons, such as salts of mercury or uranium or bacterial poisons as well. The exact mechanism for the renal changes in this experiment is obscure. It was hoped that metabolic studies might help in explaining the pathogenesis of the lesions. In the deficient animals the potassium excretion was very low during the first 3 weeks and after this period practically none of this cation was found in the urine. There was a diminished excretion of magnesium from the first to the seventh week in the potassium-deficient animals as well as a diminution in the urinary output of phosphate as compared with the normal controls. These data are difficult to interpret. Renal lesions have been reported in rats on a magnesium-deficient diet, though the various observers do not agree as to the exact renal changes. Greenberg, Lucia and Tufts [6] have described extensive calcium deposition in the renal tubules with atrophy of the tubular epithelium. We have recently been able to examine the kidneys of three rats that had been placed on a magnesium-deficient diet for 35 days. In these there was necrosis of the epithelium of the convoluted tubules. Calcified material was found in only one of the animals. The change was much more severe than we observed in the potassium-deficient animals, nor was tubular dilatation prominent. McKay and Oliver [12] have observed lesions in rats which bear a striking similarity to those we have observed by feeding an excess of inorganic

phosphate. In our animals, as previously noted, there was a diminution in the output of phosphate. The exact nature of the mechanism involved in the renal tubular necrosis in the potassium-deficient animals is obscure.

Summary

By the utilization of a diet adequate in all known respects except for an extremely low content of potassium (0.01 per cent) lesions have been produced in the heart and kidneys of rats restricted to this diet. In the heart, necrosis of the myocardial fibers followed by scarring has been described. Necrosis of the renal tubular epithelium with regeneration and tubular dilatation was also observed. The exact mechanism for the production of these lesions has not been elucidated.

Note: The technical work was performed by Miriam C. Reed and the photomicrographs were made by Milton Kougl.

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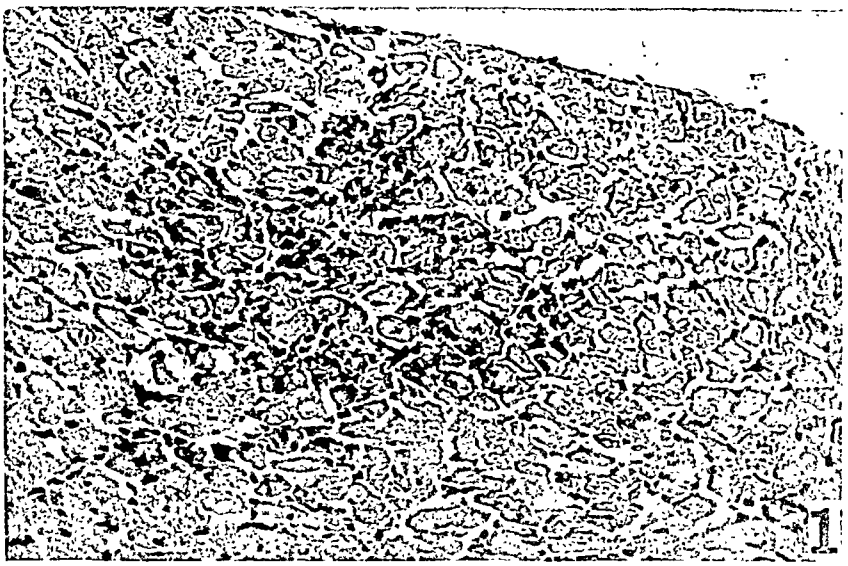
DESCRIPTION OF PLATES

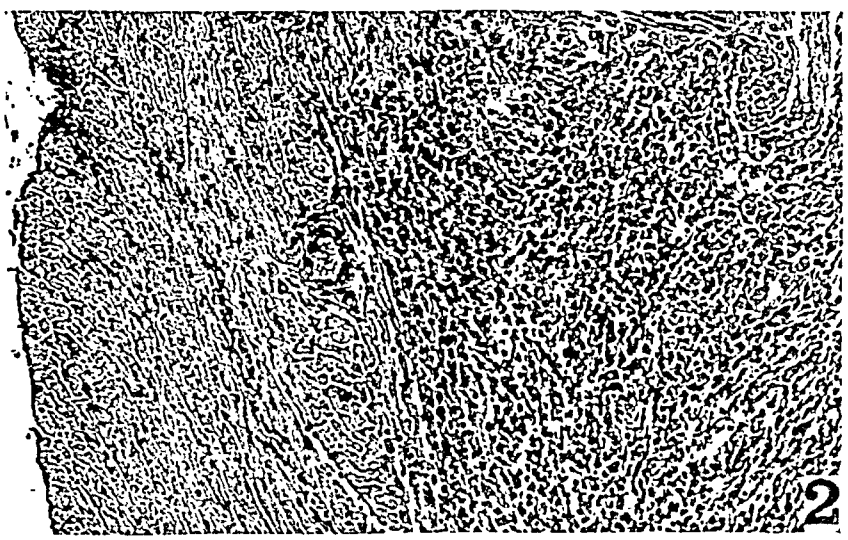
PLATE 6

FIG. 1. Fresh lesion in the myocardium of a potassium-deficient animal showing leukocytic infiltration about necrotic muscle fibers. × 400.

FIG. 2. More extensive lesion in the myocardium of a potassium-deficient rat showing diffuse mononuclear infiltration after 15 days on the diet. × 100.

FIG. 3. Photomicrograph showing scarring in the myocardium of a potassium-deficient animal after being on the diet 87 days. × 100.







Follis, Orent-Keiles and McCollum Lesions with Potassium Deficiency

PLATE 7

FIG. 4. Frozen section of kidney from a rat on the potassium-deficient diet for 13 days, showing lipid droplets in tubular epithelium (stained with Scharlach R). × 200.

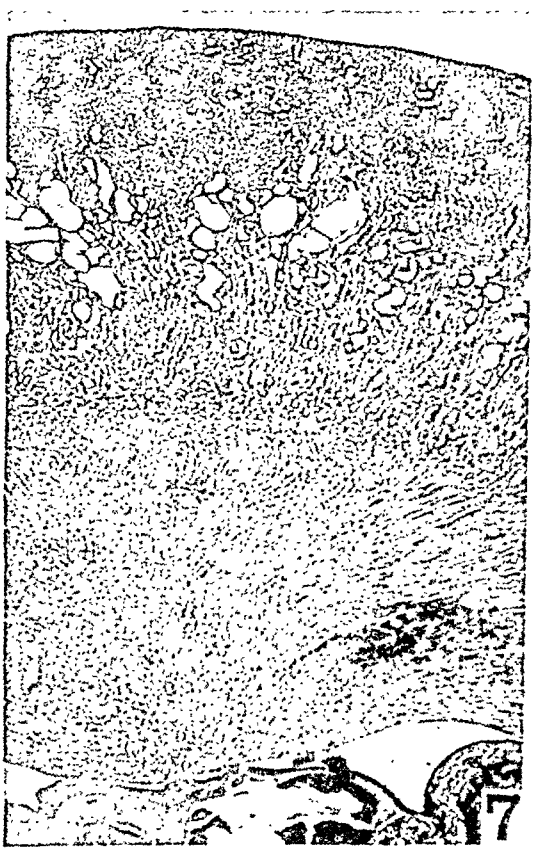
FIG. 5. Photomicrograph showing vacuolated tubular epithelial cells and a dilated tubule containing necrotic cells and débris, from a rat on the potassium-deficient diet for 15 days. × 200.

FIGS. 6 and 7. Medium and low power views of kidney from a rat on the potassium-deficient diet for 6 weeks showing dilatation of the tubules. Arrows point to small collections of calcified material in the tubules. × 90 and × 15.









Follis, Orent-Keiles and McCollum

Lesions with Potassium Deficiency



THE DEPOSITION OF CALCIUM IN THE HEARTS AND KIDNEYS OF RATS IN RELATION TO AGE, SOURCE OF CALCIUM, EXERCISE AND DIET*

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In this laboratory, stereoroentgenographs of some of the organs of our rats, taken at the time of autopsy, have proven useful in estimating the incidence and extent of calcium deposition. We have previously reported the use of this technic in connection with the study at autopsy of the rats from our retarded-growth experiments.[1,2] The high incidence of calcification which we found in some of the organs of those rats has led to the present study.

The data to be presented here were obtained from three experiments which were started during or near the end of the retarded-growth experiments.[1,2] Experiment I was one of several experiments conducted by McCay, Maynard, Sperling and Osgood [3] to study the effects of different levels of protein in the diet, and of exercise, on the life span of the rat. The complete diet of this group of rats is given in Table 3 of their paper. Here I shall mention only those variables in the experiment which seem to influence calcium deposition in the heart and kidneys. Experiment II was conducted by Bogart,[4] Asdell [5] and their co-workers to determine some of the effects of reproduction on the life span of the female rat. The diet used in this case was our stock diet.[6] Experiment III was conducted by McCay and associates and is still unpublished. The complete diet for this experiment is given in Table I.

CALCIFICATION OF THE HEART

Stereoroentgenographs of the hearts of the rats were made and when calcium was found, its approximate location was noted. Among the older rats a calcified region in the auricles was fairly common; in some cases calcification in the region of the valves could be seen, and in some, one or more regions of calcification were found in the ventricles. The three hearts shown in Figures 1, 2 and 3 are examples of calcified regions found in auricle, ventricle and valvular region respectively.

In all three experiments the incidence of calcification was found to increase with the age at death (Table II).

In experiment I,[3] in which protein, exercise and fatness were variables, stereoroentgenographs of 232 hearts were made at the time of

* Aided by a grant from the Rockefeller Foundation for research in longevity.
Received for publication, February 20, 1941.

autopsy. The average age at death for these animals was 636 days. About 42 per cent of the hearts showed at least one calcified region. Twenty-four per cent showed calcification in the auricles, 16 per cent in the ventricles and 12 per cent in the region of the valves.

The incidence of calcification of the heart in relation to the different dietary variables in this experiment showed no marked differences. However, the exercise variable combined with the level of food intake gave a result which may be significant. The hearts of 58 rats on unrestricted food intake and enforced exercise [3] showed an incidence of calcification of 55 per cent, while those of 58 rats not allowed to become fat but forced to exercise showed an incidence of only 36 per cent.

In experiment II [4] (the longevity-reproduction experiment) the average age at death for 287 females, from which hearts were obtained, was 663 days. Forty-three per cent of these hearts showed some calcification. Twenty-five per cent showed calcification in the auricles, 11 per cent in the ventricles and 11 per cent in the region of the valves.

TABLE I

Diets in Experiment III

	E	F	G	H	I	J
	(Per cent)	(Per cent)	(Per cent)	(Per cent)	(Per cent)	(Per cent)
Dry liver	12	12	12	12	12	12
Sucrose	10	10	10	10	10	10
Cellulose	2	2	2	2	2	2
Wesson oil	7	7	7	7	7	7
Yeast	5	5	5	5	5	5
Cod liver oil*	0.25	0.25	0.25	0.25	0.25	0.25
$CaCO_3$	0.33	0.33	0.33	—	—	—
$CaHPO_4 \cdot 2H_2O$	—	—	—	1.10	1.10	1.10
NaCl	0.70	0.20	1.50	0.70	0.20	1.50
Starch (cooked)	63.43	63.22	61.92	62.95	62.45	61.15

* Fortified cod liver oil (vitamin D, 800 I. U. per gm.; vitamin A, 6000 I. U. per gm.)

TABLE II

Incidence of Calcification of the Heart in Relation to Age at Death

Age range (days)	Experiment I male rats		Experiment II female rats		Experiment III male rats	
	Number of hearts	Incidence of calcification (per cent)	Number of hearts	Incidence of calcification (per cent)	Number of hearts	Incidence of calcification (per cent)
300–399	10	20	12	33	—	—
400–499	26	23	21	29	18	22
500–599	56	29	41	41	52	31
600–699	66	44	57	46	56	25
687–704*	—	—	97	38	69	49
700–799	51	50	26	35	17	47
800–899	16	75	21	62	4	50
900	7	86	12	75	—	—

* Rats killed accidentally by high temperature.

In Table III the groups of rats used in this experiment are arranged in the order of increasing incidence of calcification of the heart. The highest incidence appears in the group in which breeding was not started until the rats were nearly 300 days old, and the lowest incidence is found in the group which was castrated a short time after weaning. For details of the handling of the rats in this experiment, the paper by Bogart and co-workers [4] should be consulted.

In experiment III, where the variables were mineral supplement and exercise, stereoroentgenographs of the hearts of 216 male rats were made. The average age at death for these animals was 636 days. Here the incidence of cardiac calcification was 36 per cent. The auricles showed an incidence of calcification of 18 per cent; the ventricles, 13 per cent, and the valvular region, 9 per cent.

The results of this experiment suggest that the form of the mineral supplement affects the incidence of calcification. The hearts of 108 rats receiving the $CaCO_3$ supplement showed an incidence of calcification of 30 per cent and those of 108 rats receiving the $CaHPO_4 \cdot 2H_2O$ supplement an incidence of 43 per cent.

Half of the animals receiving the $CaCO_3$ supplement and half of those receiving the $CaPHO_4 \cdot 2H_2O$ supplement were given moderate enforced exercise.[3] The exercise seemed to have had no effect on the incidence of calcification of the heart among the 108 rats receiving the $CaCO_3$ supplement. In this group the incidence was 30 per cent for the 54 animals forced to exercise and 30 per cent for the 54 animals not forced to exercise. However, in the case of the 108 rats receiving the $CaPHO_4 \cdot 2H_2O$ supplement, the results suggest an exercise effect. The hearts of 54 rats from this group that received no enforced exercise showed an incidence of calcification of 57 per cent and those of the 54 rats with enforced exercise an incidence of only 27 per cent.

These two diets were analyzed for calcium and phosphorus. The diet containing the $CaCO_3$ supplement contained 0.25 per cent of cal-

TABLE III

Incidence of Calcification of the Heart in Longevity-Reproduction Experiment (Experiment II)

Group	Number of hearts	Average age at death	Incidence of calcification
		(days)	*(per cent)*
Castrates	50	666	33
Virgins	48	652	35
Bred normally	47	643	40
Gestation without lactation	47	652	47
Bred early	45	683	49
Bred late	48	681	52

cium and 0.27 per cent of phosphorus (by weight), giving a calcium-phosphorus ratio of 0.93. The other diet contained 0.38 per cent calcium and 0.59 per cent phosphorus, with a calcium-phosphorus ratio of 0.64.

Calcium Deposition in Kidneys

Figures 4, 5 and 6 show different types of renal calcification which have been found in rats in these experiments. Figure 4 shows rather extensive calcium deposition in the medulla of the kidney; Figure 5, a deposition in the pelvis and Figure 6, extensive calcification in the cortex.

In experiment I, stereoroentgenographs of 468 kidneys from 234 male rats showed 48 kidneys with calcified regions sufficiently large to be detected by this method. This gives an incidence of 10.3 per cent. The extent of this calcification varied from one small spot in the kidney to a few cases as extensive as those in Figures 4, 5 and 6. In this experiment 5 per cent of the kidneys showed calcification in the cortex of the kidney, 4 per cent in the medulla and 2 per cent in the pelvis-pyramid region.

If the different variables in the experiment are considered, the lowest incidence of renal calcification (4 per cent) is found in the group of 57 rats with restricted food intake and no enforced exercise, while the group showing the highest incidence (17 per cent) was a group of 58 rats unrestricted in food intake and forced to exercise.

Considering the dietary variables only, the lowest incidence of renal calcification (7 per cent) is found in a group of 57 rats on the high casein diet, and the highest incidence (16 per cent) in a group of 55 rats on the low-liver diet.

There is also a suggestion that the age at which the rat was shifted from the stock diet to the experimental diet had an effect on the incidence of renal calcification. The kidneys of 238 rats started on this experiment between the ages of 204 and 280 days showed an incidence of calcium deposition of 6 per cent, while the kidneys of 230 rats started on the experiment between the ages of 420 and 431 days showed an incidence of 15 per cent.

In experiment II (longevity-reproduction), in which all of the rats had the stock diet,[6] the incidence of calcium deposition in the kidneys was low and the calcification was less extensive than in the kidneys of the rats of either of the other experiments. In this case stereograms were made of the kidneys of 283 female rats. Of the 566 kidneys examined, only 50 (9 per cent) showed any detectable calcium deposition. Two per cent of the kidneys showed calcium in the pelvis-pyramid

region, 4 per cent in the medulla and 3 per cent in the cortex. A comparison of the groups subjected to the different reproduction variables showed no marked differences.

In experiment III an examination of the stereograms of 432 kidneys from male rats showed an incidence of calcification of 18 per cent. Four per cent showed small calcium deposits in the pelvis-pyramid region, 13 per cent showed calcification in the medulla and 4 per cent showed some calcification in the cortex.

The exercise variable in this experiment seemed to have had no effect on calcium deposition in the kidneys. However, the mineral supplement variable had affected the calcium deposition in the kidneys. Of 216 kidneys from rats receiving the $CaCO_3$ supplement, only 9 per cent showed any calcium. Of the 216 kidneys from rats receiving the $CaHPO_4 \cdot 2H_2O$ supplement, 28 per cent showed calcium deposits.

SUMMARY

1. This study shows, in all three experiments, that the incidence of calcification of the heart is an increasing function of the age at death.

2. The auricles are shown to be more common sites for calcium deposition than are the ventricles and the region of the valves.

3. Rats not allowed to become fat and forced to exercise show a lower incidence of calcification of the heart than those on unlimited food intake and enforced exercise.

4. The results suggest that the form of the mineral supplement has an effect on the incidence of calcification of the heart and that moderate enforced exercise may modify this effect.

5. In a large group of female rats all receiving the same diet but subject to reproduction variables, the highest incidence of calcification of the heart was found in a group in which breeding was delayed until middle life.

6. In the study of the kidney the medulla was found to be the most common site for calcium deposition.

7. Rats receiving a mineral supplement of $CaHPO_4 \cdot 2H_2O$ showed an incidence of renal calcification three times that shown by rats receiving a supplement of $CaCO_3$.

NOTE: The author is indebted to C. M. McCay and L. A. Maynard for helpful suggestions, to Gordon Ellis for calcium and phosphorus analyses of the diets, and to Gladys Sperling for help with the stereoroentgenographs.

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DESCRIPTION OF PLATE

PLATE 8

Roentgenographs of hearts and kidneys

FIG. 1. Heart, showing calcium deposition in auricle.

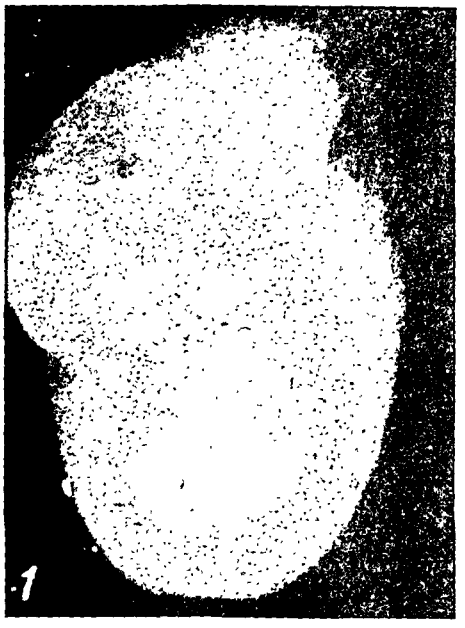
FIG. 2. Heart, showing calcium deposition in ventricle.

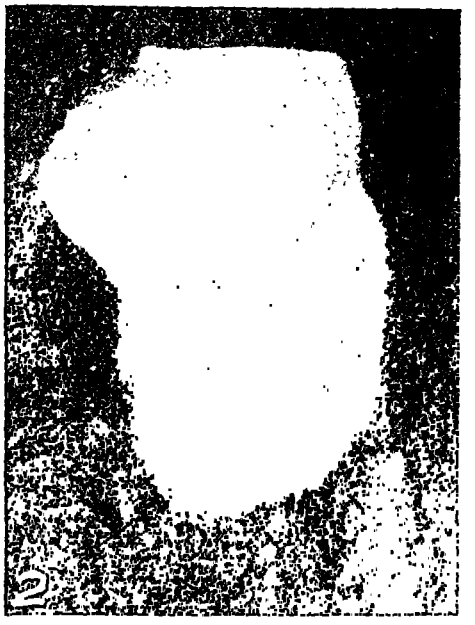
FIG. 3. Heart, showing calcium deposition in valvular region.

FIG. 4. Kidney, showing calcium deposition in medulla.

FIG. 5. Kidney, showing calcium deposition in pelvis.

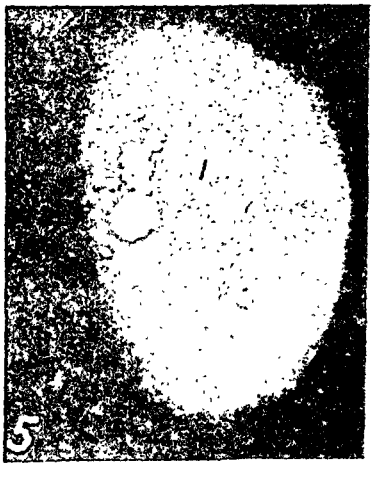
FIG. 6. Kidney, showing calcium deposition in cortex.


1


2


3


4


5


6



PATHOLOGICAL CHANGES IN NUTRITIONAL GASTRITIS IN RATS *

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In 1913, Fibiger [1] described lesions in the rumen of the rat's stomach which he attributed to infestation with a nematode, Spiroptera neoplastica (*Gongylonema neoplasticum*). Subsequent investigators discovered similar changes in the stomachs of rats free of parasites and expressed with increasing definiteness the conviction that they were dealing with the result of nutritional deficiencies. According to present-day standards, the white bread diet used by Fibiger [2] for his experimental animals was certainly inadequate in many respects.

Fibiger's original description was restricted to alterations in the rumen and succeeding experimenters continued to center their attention on the non-secretory portion of the stomach. From the standpoint of man and related species which do not have a rumen, experimental studies dealing with the secretory part of the rat's stomach are of wider interest and applicability than observations on the forestomach. Some investigators mentioned changes in the secretory epithelium when the rumen was affected, and in part described them. Howes and Vivier[3] gave a better account of these lesions than their predecessors, but they too recorded relatively few instances of abnormalities in the glandular mucosa in comparison with the occurrence of lesions in the forestomach. Howes and Vivier pointed out the similarity of the changes in the secretory and in the non-secretory epithelium.

It has been my experience that when the stomach is examined in the stretched-out condition, lesions are observed with greater regularity in the antrum than in the rumen, and within the glandular region there is a marked difference regarding frequency and type of lesion between antrum and fundus. The antrum is also distinguished from the other components of the stomach by the presence of lymphoid tissue. Histological study of the antral lesions by means of serial sections gives an insight into the nature of the pathological process in the mucosa.

This report is descriptive in character and deals with the pathological changes occurring in the different parts of the rat's stomach in nutritional gastritis. The dietary deficiencies concerned in the development of gastric lesions and details regarding nutritional effects will be discussed in another paper.

* Received for publication, March 15, 1941.

Methods

Seven hundred and twenty albino rats of both sexes were placed upon inadequate diets at 28 days of age. Various kinds of deficient diets were employed. One was the Pappenheimer and Larimore [4] diet consisting of a mixture of flour and salt. Another was the casein and starch diet used by Dalldorf and Kellogg,[5] Sure and Thatcher [6] and others. Several modifications with relation to protein, mineral salts and the B complex were made in the different diets. The feeding experiments were terminated at the end of 4 weeks when the animals were killed and their stomachs examined for lesions. As controls, 116 rats of both sexes and 56 days of age were examined after being kept for 4 weeks on a good stock diet.

The rats were killed with ether and complete autopsies were performed. The stomachs were removed immediately and opened along the greater curvature. After washing in normal saline solution, they were stretched by means of pins on a piece of cardboard and immersed in a 4 per cent solution of formaldehyde. After an interval of 24 hours, the specimens were examined for gross changes in the mucosa. Many lesions that were not seen in the fresh state became visible after formaldehyde fixation.

For histological examination, tissues were imbedded in paraffin and sections were stained with hematoxylin and eosin. In many instances serial sections of lesions were made. For control study, the entire antrum of a normal rat, 56 days old, was cut serially.

Gross and Microscopic Anatomy of the Stomachs of Rats Fed a Stock Diet

The rat's stomach is divided into two parts by a transverse, firm, white, raised ridge. The proximal portion is the rumen or forestomach which occupies about two-fifths of the total area of the stomach in the stretched condition. It has a thin, grayish white, translucent wall and a smooth, non-secretory internal surface. The esophageal orifice is just above the ridge on the lesser curvature. The portion between the ridge and the pylorus is the secretory or glandular area which in turn is subdivided into antrum and fundus. When the stomach is opened along the greater curvature, the antrum appears as a roughly semicircular area occupying the entire lesser curvature below the ridge. The antral wall is thin and translucent, and the lining is slightly roughened. The fundus includes the entire greater curvature below the ridge, except for a short distance above the pylorus. It has a thick, opaque wall and a velvety, furrowed surface. The border between antrum and fundus is demarcated by an abrupt elevation of the mucosa.

The rumen and dividing ridge are lined by a stratified squamous epithelium which is continuous with the epithelial lining of the esophagus. The mucosa is composed of a superficial thin layer of keratinized cells beneath which there is a layer of cells containing eleidin granules, and a deeper zone where the cells are nucleated. The mucosa is separated from an underlying, well developed muscularis mucosae by a

thin layer of connective tissue, which contains an occasional lymphocyte or eosinophilic polynuclear leukocyte. Another narrow band of loose connective tissue intervenes between the muscularis mucosae and the muscularis which is composed of inner circular and outer longitudinal fibers. At the limiting ridge, the epithelium is thickened and elevated, and at its junction with the glandular portion of the stomach there is a transition from the squamous to the columnar type of cell: Here, the submucosa is composed of dense connective tissue which is infiltrated by variable numbers of wandering cells and often contains glandular acini.

In about 15 per cent of all animals, one or more discrete, hard, white, raised spots measuring 1 to 2 mm. in diameter are found on the ridge or in the glandular region immediately adjacent to the ridge where the latter dips below the esophageal opening. Microscopically, such an area is a papillomatous projection of hyperplastic squamous epithelium continuous with the glandular mucosa and supported by a connective tissue stroma. All of the components of the gastric wall become thickened at the junction of the esophagus with the rumen.

The mucosa of the fundus consists of long, narrow, tubular glands lined by numerous large, round, prominent parietal cells and relatively inconspicuous chief cells. In the antrum, the glands are shorter and wider than in the fundus and are lined by columnar epithelium. Mitoses are not uncommon. The transition from fundic to antral mucosa is fairly abrupt and occupies an area of 300 to 500 μ. The glands are supported by a loose connective tissue stroma containing a few lymphocytes and polynuclear eosinophils. Brunner's glands extend into the antrum beneath the muscularis mucosae for a short distance above the pylorus. The muscularis mucosae and muscularis are continuous with the same structures in the rumen and are separated from one another by a layer of fine connective tissue.

Lymphoid tissue is found only in the antrum and exists in the form of large and small nodules and as diffuse lymphocytic infiltration. In control rats, large nodules occupy definite sites in the antrum. They are found in the midline just below the limiting ridge or along the fundic borders, more often on the left side than on the right. As a rule they exist singly. In rare instances two or even three may be present.

A large nodule appears as a circumscribed oval or round elevation of the mucosa measuring 2 to 4 mm. in diameter. It has a round, linear, or Y-shaped central depression. Microscopically, there is in the submucosa a sharply defined, nonencapsulated, densely packed collection of small lymphocytes. Germinal centers may be present. The overlying epithelium often shows at the fundic borders a transition from the antral to the fundic type of cell and is raised above the level of the neighboring mucosa. When a nodule exists just below the ridge, the overlying glands are sometimes hyperplastic. In the region of the central depression, the glands become short and shallow or are converted into a single flat layer of epithelium. The muscularis mucosae loses its continuity at the margins of the nodule which extends to the muscularis.

Large lymphoid nodules are of considerable interest and are being studied from two standpoints. One problem is concerned with whether they are normal structures corresponding to the lymphoid nodules in the small intestine[7, 8] or whether they are a manifestation of a nutritional deficiency in the stock diet. The other line of investigation deals with the incidence of nodules in gastritis. This phase of the study is complicated by the fact that it is difficult to distinguish, grossly, nodules from ulcerative lesions.

Small lymphoid nodules cannot be identified in the gross specimen. They vary considerably in different rats with regard to number, size and distribution. The nodules are oval in shape and measure from 30 by 80 μ to 120 by 180 μ. They are found most frequently near the midline or along the fundic borders, and consist

of localized, dense aggregates of small lymphocytes in the submucosa. Germinal centers are not present.

As a rule, diffuse lymphocytic infiltration in the submucosa is not encountered in control animals except in the prepyloric region; but in nutritional gastritis, infiltration is found frequently in association with mucosal lesions.

Mucosal Changes in Nutritional Gastritis

Antrum

The essential pathological change in the antrum consists of a break in the continuity of the surface lining of the mucosa in a region of epithelial hyperplasia. In the gross specimen there is a well defined, round, raised area measuring 2 to 4 mm. in diameter with a central depression which may be filled with a white or red plug. Instead of appearing as a small pit, in larger lesions the center may be an oval defect, or a linear or Y-shaped groove. There are marked variations in different animals with regard to the incidence and number of grossly visible ulcerations. The lesions are usually multiple and, in order of frequency, are found in the prepyloric region, on the lesser curvature and along the fundic borders. The distribution often corresponds to the course of the main gastric blood vessels.

The initial phases of the development of epithelial defects are not seen very often, even with the aid of serial sections. The earliest microscopic change is a minute break in the lining epithelium with an acute inflammatory reaction in the submucosa. Fibrinous edema, capillary dilatation and congestion, and leukocytes of the polynuclear variety appear in the loose connective tissue stroma. Polynuclear leukocytes are also found within the overlying epithelial cells, some of which show necrosis with a resultant break in continuity of the surface lining. Early changes are found in relatively normal mucosa, in areas of epithelial hyperplasia and at the sites of small and large lymphoid nodules.

Ultimately, one or more glands break down and the mucosal defect or ulcer involves the entire thickness of the mucosa and submucosa (Figs. 1 and 2). Ulcerations also occur in the epithelium covering large lymphoid nodules. Sometimes two lesions develop simultaneously and fuse to form a single large one. In some ulcers the remnants of necrotic glands are still visible in the base. In others, the defect extends to the muscularis mucosae which is infiltrated with polynuclear leukocytes. Occasionally the muscularis forms the floor. The crater is usually filled with an exudate composed of fibrin, leukocytic fragments, necrotic epithelium and red blood cells. Fresh hemorrhage may be present. The subjacent loose connective tissue is edematous and is invaded by numerous wandering cells, chiefly lymphocytes. The margins of the ulcer, which are abrupt and often undermined, are formed by hyperplastic glands whose lining cells frequently contain mitoses.

Areas of epithelial hyperplasia occur, particularly in the prepyloric region, without a demonstrable break in the mucosa. Serial sections of such areas often reveal central depressions containing short atypical glands which may represent the sites of healed ulcerations. Sometimes the elongated glands are tortuous and dilated, and the cytoplasm of the lining cells is vacuolated. The underlying loose connective tissue layers may be edematous and infiltrated by small lymphocytes.

When ulcerations are examined by the serial section method, it becomes evident that repair and injury go on simultaneously and that the pathological process is a continuous one. In many instances it can be shown in the same lesion that the mucosal defect is being covered with new epithelium in one place while necrosis and inflammation are active in another (Figs. 2 and 3). Various stages of injury and repair are also demonstrable in different lesions within the same antrum. At one site there may be fresh necrosis while at another there is no longer evidence of active breaking down of the mucosa and the defect is bridged by an uninterrupted layer of new epithelium. Later stages of repair are also observed where regenerating glands are filling in the craters of ulcers (Fig. 4). With complete glandular restoration, the site of a previous ulceration may not be identified or may appear as a depression in an area of hyperplasia.

However, healing does not always go on to complete regeneration of the mucosa. Instead, the newly formed epithelium may again break down and a lesion of chronic type develop as a result of recurrent injury and repair. Grossly, such an ulcer has a large crater and heaped-up, rounded edges. Microscopically, the floor of the defect is covered by a single layer of epithelium of recent origin, but the continuity of the new surface lining is broken by areas of fresh necrosis and hemorrhage. In the base of the ulcer there are a few short, atypical glands, young proliferating fibroblasts and capillaries. The crater contains fibrinopurulent exudate and blood clot. At the margins there are distorted hyperplastic glands and many of the epithelial lining cells are vacuolated. Figure 5 shows a large ulcer at the site of a large lymphoid nodule. The substance of the nodule is replaced to a great extent by young vascular connective tissue infiltrated by numerous eosinophils. The surface of the mucosal defect is incompletely covered by newly regenerated epithelium.

Rumen

The pathological changes in the mucosa of the rumen are essentially of the same nature as those in the antrum except that squamous instead of glandular epithelium is involved in the process. Lesions are found most frequently at the esophageal orifice and along the limiting ridge;

sometimes they are distributed over the entire surface of the rumen. Their number, size and shape are extremely variable and they tend to become confluent. As a rule, the lesions appear as discrete, irregularly round or oval, firm, mucosal elevations with central brownish depressions; or they may have a linear or stellate appearance. Sometimes cauliflower-like or papillomatous growths are present. The dividing ridge is frequently thickened and nodular, especially near the esophageal opening.

Microscopically, epithelial hyperplasia is particularly prominent and varies from a simple thickening, with rounded processes extending down to the muscularis mucosae, to long papillary outgrowths projecting from the surface. Mitoses are frequently seen. In the submucosa, leukocytic infiltration and dilatation of blood vessels often accompany marked hyperplasia. The muscularis mucosae and the muscularis may be hypertrophied.

Superficial necroses and deeper ulcerations occur in areas of epithelial hyperplasia. In a typical acute ulcer there is a complete break in continuity of the hyperplastic epithelium which forms the margins of a deep crater (Fig. 6). The floor of the defect is covered with necrotic epithelial cells and fragmented leukocytes. The subjacent submucosa and the layer of loose connective tissue between the muscularis mucosae and muscularis may show edema, capillary dilatation and cellular infiltration. The wandering cells are chiefly polynuclear eosinophils, but lymphocytes and mononuclear leukocytes are also present. Early healing is rarely seen but occasionally a single layer of young epithelial cells may be observed growing in from the edges and incompletely covering a mucosal defect.

Cyst formation also occurs in areas of epithelial hyperplasia. The cysts are irregularly shaped and extend through the muscularis mucosae down to the muscularis. Near the surface they are lined by hyperplastic squamous epithelium, but deeper there is a transition to low columnar epithelium. Numerous, smaller, glandlike structures lined by the same type of columnar epithelium accompany the larger cysts and invade the muscularis.

Fundus

Lesions in the fundus are uncommon even when the antrum and rumen show extensive pathological changes. The characteristic gross finding is mucosal hemorrhage in the form of multiple, pin-point petechiae or large, irregular, streaky blood clots. The bleeding is often so brisk that the entire gastro-intestinal tract is found at autopsy to be filled with fresh blood. Histologically, the hemorrhage is accompanied

by glandular necrosis, which is usually superficial but may involve the entire thickness of the mucosa. Epithelial hyperplasia is absent.

Gross and microscopical examination of the small and large intestine in a large number of the animals on deficient diets revealed no ulcerations or hyperplasia of the mucosa.

The following results were obtained in a group of 186 rats with gastric changes fed on a diet low in protein and deficient in the B complex: in 66, lesions appeared only in the antrum; in 30, abnormalities were present only in the rumen, and in 90, ulceration and hyperplasia occurred in both antrum and rumen. The fundus was the seat of hemorrhage in 19 animals but always in association with other lesions in the stomach.

Summary

In nutritional gastritis, the antrum and rumen may be involved separately or together. The antrum shows changes more often than the rumen. The fundus remains relatively free of lesions.

Lymphoid tissue is found only in the antrum. The significance of large lymphoid nodules is discussed.

The pathological changes in the mucosa of the antrum consist of ulcerations in areas of epithelial hyperplasia that tend to undergo spontaneous healing. Since various stages of injury and repair are found to exist simultaneously in the same lesion and in different lesions, it appears that the process is continuous. Recurrent necrosis and hemorrhage develop in healing defects and lead to chronic changes.

In the rumen, the mucosal abnormalities are essentially of the same nature as in the antrum except that hyperplasia is more striking in the rumen while healing of epithelial defects is more evident in the antrum.

The lesions in the fundus differ from those in the antrum and rumen and are essentially hemorrhagic erosions.

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DESCRIPTION OF PLATES

All illustrations were prepared from sections from the stomachs of rats on diets low in protein and deficient in the B complex. Hematoxylin and eosin stain.

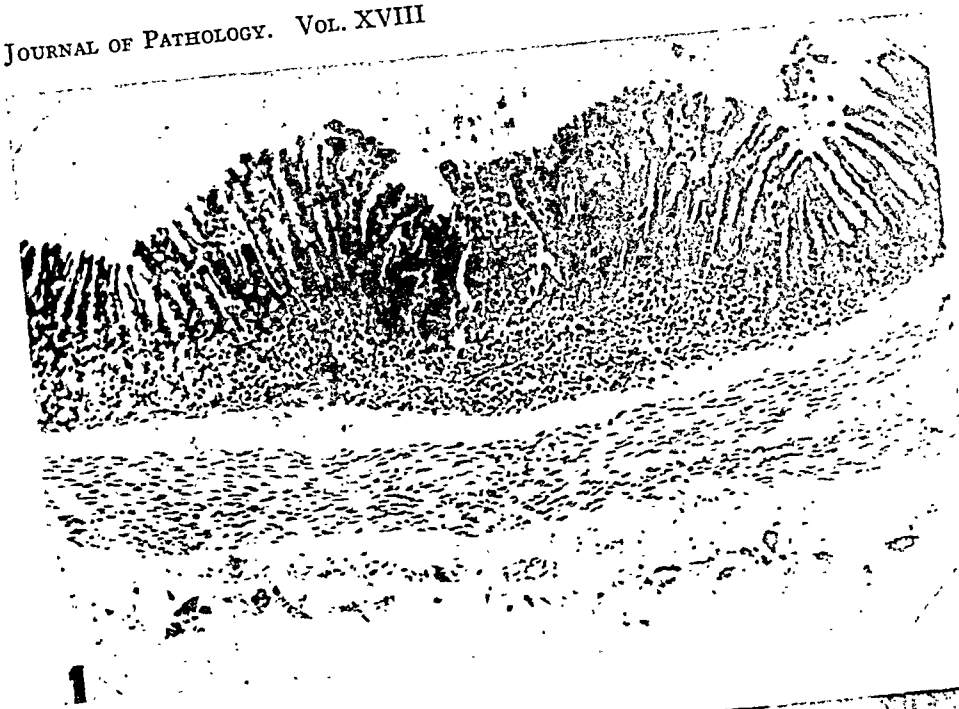
PLATE 9

FIG. 1. Antrum. An ulcer in an area of epithelial hyperplasia. ×110.

FIG. 2. Antrum. High power magnification of the ulcer in Figure 1. × 373.

FIG. 3. Another section of the same lesion showing repair. The base is covered with regenerated epithelium. × 373.

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PLATE 10

FIG. 4. Antrum. New gland formation in the base of a healing ulcer. × 95.

FIG. 5. A large ulcer with chronic changes at the site of a large lymphoid nodule. The base of the ulcer is partly covered with regenerating epithelium. × 95.

FIG. 6. Rumen. An ulcer in an area of epithelial hyperplasia. A single layer of proliferating epithelial cells is beginning to bridge the defect. × 230.







Berg Nutritional Gastritis i

THE DISTRIBUTION OF INTIMAL ATHEROMATOUS LESIONS IN THE ARTERIES OF RABBITS ON HIGH CHOLESTEROL DIETS *

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Most of the numerous theories which have been advanced to account for the focal nature and usual distribution of early aortic atheromatous lesions are based on the assumption that the affected areas have been subjected to greater "stress and strain" in a selective fashion. This is said to lead to impairment of elasticity followed by fibrous overgrowth of the intima locally, or wrinkling with formation of connective tissue interstices (Duguid,[1] Moschcowitz[2]); swelling of the "ground substance" (Aschoff[3]); separation or actual intimal "herniation" (Krafka[4]); local anemia (Moon[5]); increased infiltration (Anitschkow[6]); or increased intimal vascularity and hemorrhage (Winternitz, Thomas and LeCompte[7]). Many of these theories are reviewed briefly by Krafka. All of them postulate a preliminary, preparatory alteration which induces the subsequent deposit of lipids.

In most instances, by the time fibrous proliferation has occurred in intimal plaques, lipids are present as well, so that it is impossible to be certain that the fibrous overgrowth is not entirely a secondary reaction to them. Even if lipid is not demonstrable, there is no assurance that it was not formerly present and has been reabsorbed. It is commonly believed that the lipidic streaks seen in intimas early in life are capable of reabsorption and disappearance. For example, the valvular deposits seen in infants are less frequently observed in older children (Aschoff,[3] Zinserling[8]) and this has been interpreted as indicating that such early deposits are not always permanent. If this is true, there is no reason why early accumulations of lipids elsewhere, such as in the vicinity of branch orifices, should not be equally reversible.

In a previous study of the postmortem elasticity of the adult human aorta, I[9] was able to confirm the observation of others (notably Duguid[1] and Moschcowitz[2]) that intimal plaques tend to occur in greatest number and size in those parts of the aorta which are least movable because of the vessel's attachments. It was also found that regardless of the amount of intimal plaque formation, such fixed areas of all aortas lose their extensibility equally and progressively with advancing age, and earlier and more rapidly than the more freely movable parts of the vessel.

* Received for publication, April 5, 1941.

From these findings, I advanced the hypothesis that intimal lipids, whether extracellular or contained within mobile phagocytes, tend to converge or migrate as a result of the pulse movements of the vessel towards points of least mobility, being impeded in their progress at points where firm scars have fused the media to the intima, obliterating potential tissue spaces. This might account for the accumulation which occurs, for example, at the scar of the obliterated opening of the ductus arteriosus. Similarly, progress might be arrested at points where arterial branches form sharp angles at their origins such as at the mouths of the intercostal arteries. The formation of discrete deposits at definite points in the aorta, according to this interpretation, would be the result of such intra-intimal convergence of lipids. In other words, regardless of the manner in which lipids may enter the intima—whether as a finely dispersed deposit directly from the lumen through the endothelium or from penetrating vasa vasora—their final resting places are not necessarily identical with the points of entrance.

To test this, it would be of interest to know if lipids deposited in the arteries of rabbits fed on high cholesterol diets undergo intramural adjustments in position. The basic disturbance which leads to the deposition of cholesterol compounds in the arteries of rabbits maintained on such diets may be radically different from that which obtains in man. Persistent hypercholesteremia and widespread lipidic deposits in extravascular sites, as has been frequently pointed out, are not associated with the human arterial lesions. Nevertheless, the arterial lesions of the rabbit are the closest approximation to the early human ones that have yet been achieved experimentally. Not only in their character but also in their distribution there are many striking similarities. This is especially true of the tendency of both to occur at the mouths of arterial branches. The only important discordant feature in distribution is the paucity of lesions in the abdominal portion of the rabbit's aorta as compared to the proximal portion. The reverse is the usual finding in man. However, it seems reasonable to assume that the conditions which lead to localization of lipids at the mouths of arterial branches in one species are also in effect in the other.

A number of studies have been reported on the relation of lipidic deposit to points of fixation in the arteries of rabbits fed high cholesterol diets. Anitschkow [10] observed that the intima, overlying the edges of medial lesions produced by injections of adrenalin, was frequently the seat of deposit of lipids following cholesterol feeding. Schilling [11] injured the rabbit's abdominal aorta under similar conditions. He failed to find cholesterol deposits at the actual site of injury but in one animal described a large deposit just proximal to the damaged area which he

believed was related not to the injury but to an arterial orifice in that region. However, large deposits of lipids at this distal point are unusual. In my opinion an alternative explanation for both this finding and for that of Anitschkow would be that lipid had collected at the margin of the injured areas but had failed to penetrate into the actual site because the reaction to the injury had caused firm union of the intima and media at this point.

Ssolowjew [12] made a more extensive study of this type. He injured arteries of rabbits by cautery before and during feeding with cholesterol. When the vessel was injured after hypercholesteremia had already been established, cholesterol was found in the actual field of injury and both intima and media were heavily impregnated. When the injured area was allowed to heal for 50 to 92 days before cholesterol feeding was instituted, the site of cauterization itself was devoid of lipids but a ring of cholesterol had accumulated around the margins in the intima. These findings I interpret as follows: When the vessel wall has been recently injured a local area of spasm, or at least of noncontractility, would serve as an immobilized zone and favor the collection of lipids from adjacent intimal tissue. The wall of the vessel itself being partly necrotic and inflamed, the lipids would permeate readily into the media through damaged elastic lamellae and smooth muscle cells, assuming that these, when viable and intact, offer an impassable barrier to intimal lipid. If, however, the injury had healed by fibrous tissue replacement before the blood cholesterol became increased, the site of previous damage would still be relatively non-contractile and immobilized but now lipids converging on the area would be unable to penetrate the dense fibrous tissue and would remain in the intima at its edges.

Harrison [13] caused medial calcification of the aortas of rabbits by administering large amounts of irradiated ergosterol prior to and during cholesterol feeding. He observed intimal deposits of lipids only in the uncalcified portions of the vessel and concluded that only the more mobile areas were susceptible to atheromatous formation. In his own descriptions and illustrations he shows that the intima overlying and adjacent to the margins of medial plaques of calcium contains the largest aggregates of lipids. This I again interpret as indicating that the lipids had penetrated the intima only at normal, uncalcified areas and had secondarily accumulated at the periphery of calcified, fixed areas. Failure to extend above the calcium masses would be due to th fibrosis, associated with the calcification, resulting in impenetrat fusion of the intima to the underlying tissue in these areas. is supported in another study of aortic calcification in hyper-

D by Duguid [14] in which secondary intimal changes of a proliferative type were described at the edges of the medial lesions. Later, Harrison [15] observed that rabbits' femoral arteries, rendered relatively relaxed, flaccid and presumably less mobile by previous sympathectomy, were more often and more severely involved by lipid deposits than the intact arteries of the opposite extremity.

It is difficult to devise a procedure which will immobilize portions of the arterial system without otherwise altering the same areas. If an artery is traumatized, its permeability to fluids and its adventitial blood supply are also changed. Even sympathectomy, as performed by Harrison,[15] while not directly injuring the femoral artery since the operative procedure involved removal of the second, third and fourth lumbar ganglia transperitoneally, may nevertheless lead to secondary structural changes dependent on denervation. Consequently, the results of all such experiments are open to more than one interpretation.

Lacking a method to immobilize a considerable portion of an artery without also injuring it, the next best procedure to test the hypothesis of intramural migration of lipids to areas of fixation, would be to devise a standard method of immobilizing a measured segment of an artery so that the attendant structural changes are approximately the same in all cases. Under such circumstances any differences in deposit of lipids at these sites would have to be accounted for on some other basis than the injury itself. The experimental data in this report record an attempt to accomplish this objective.

Materials and Methods

Nine adult rabbits, weighing about 2.5 Kg. each, were fed 1 gm. of cholesterol daily for a period of from 9 to 12 weeks according to the method recommended by Harrison.[15] The cholesterol was dissolved in 6 cc. of hot olive oil with enough bran added to absorb the oil.

Two days before cholesterol feeding was started, under nembutal anesthesia, cylindrical silver cuffs were placed on both common carotid and both femoral arteries. The cuffs were fashioned from sheet silver, 0.5 mm. in thickness, by cutting rectangular pieces 1.5 cm. in length and either 1.0 or 0.3 cm. wide. These were molded around cylindrical metal rods, of diameters from 0.1 to 0.4 cm., with a vise and pliers. Where the two edges came together to complete the hollow cylinder, the overlapping portions were reflected backward radially to the curved portion to form two flaps by which the cylinder could be opened or closed. These flaps were trimmed to 0.2 cm. in length and all edges were rounded by buffing. The cuff was opened sufficiently to admit the artery and closed afterwards with a heavy hemostat. The artery

was dissected free only at the actual point where the cuff was introduced. In every instance cuffs were selected with an internal diameter which was approximately equal to the external diameter of the artery so that the cuff fitted snugly, but without compression.

In five rabbits the cylindrical cuffs used were 1.0 cm. in length and either one or two of these were placed on each artery. In the other four rabbits, cuffs 0.3 cm. in length were used and from one to three were installed on each artery at varying distances. By measuring the distance of the cuffs from each other and from some fixed point in the surrounding tissue, it was determined that they were not displaced from their original location during the course of the experiment. Four other rabbits which were not fed cholesterol had similar cuffs placed on the common carotid and femoral arteries. These control rabbits were sacrificed and the vessels excised after 2, 6, 9 and 12 weeks. The reaction to the cuff itself was studied under a compound dissecting microscope and histologically.

At the conclusion of the experiment the cholesterol-fed animals were sacrificed by exsanguination and the arteries examined. Some were stained grossly with Sudan IV and fixed in a 10 per cent solution of formaldehyde to show the distribution of intimal lipid in relation to the sites of the cuffs. Others were examined unfixed and unstained in 0.9 per cent saline and subsequently fixed in Zenker's solution without addition of acetic acid. The entire vessel was embedded and sectioned serially. Every tenth section was stained alternately with hematoxylin and eosin and with combined Weigert and van Gieson stains.

All of the nine rabbits fed cholesterol exhibited the usual lipidic deposits but to a varying degree in the liver, adrenals, spleen, kidneys, aorta and pulmonary artery.

Results

Reaction of Arteries to Silver Cuffs

The silver cuffs became enveloped in a layer of fibrous tissue which was continuous with the adjacent adventitia at the ends of the cuffs. The thickness of this fibrous layer was considerably greater when the cuffs were 1.0 cm. than when they were 0.3 cm. long. In the case of the carotid arteries, this fibrous covering formed a distinct sheath or envelope which was only loosely attached to the surrounding fascia. The fibrous growth about the cuffs on the femoral arteries, however, extended more widely and was firmly united to adjacent structures, including neighboring veins, nerve trunks and muscle sheaths. When the fibrous sheaths were incised and cut away, the cuffs were found to be loosely held within them and could be readily slipped off o

artery. Between the cuff and the underlying adventitia there was no union, although the adventitia was thickened by connective tissue proliferation which was continuous at the cuff's edges with the fibrous mass which covered the cuff externally. The surface of the silver cuffs was coated with fine brownish black precipitate which stained the adventitia and all the opposing surfaces of connective tissue as well. Sometimes this discoloration was visible through the intimal surface of the vessel. The arterial wall within the cuff was intact but thin. It was quite inelastic but still possessed considerable tensile strength. The intima within the 1.0 cm. cuffs often had a finely wrinkled or corrugated appearance, but that within the smaller 0.3 cm. cuffs was quite smooth.

Microscopically, the fibrous overgrowth in the adventitia consisted of irregularly disposed bands of collagen with collections of lymphocytes, macrophages and small capillaries. In arteries examined 2 weeks after application of the bands, cellular infiltration and capillary proliferation were more conspicuous and fibrous tissue formation less pronounced, but no suppuration or necrosis of tissue was observed. Extravasated erythrocytes and polymorphonuclear leukocytes were sometimes seen in small numbers. After 9 to 12 weeks the fibrous masses were largely avascular but still contained collections of lymphocytes and phagocytic mononuclear cells. The latter frequently contained a brownish, amorphous, granular pigment, the bulk of which failed to give the Prussian blue reaction for inorganic iron and probably was derived from the silver. Multinuclear foreign body giant cells were rarely encountered. The adventitia of the artery was thickened at the edges and within the cuffs. The media was thinned out to two-thirds or one-half of its normal thickness. This was more pronounced when 1.0 cm. cuffs had been used. The thinning began abruptly at the edges of the cuff and was quite uniform along its length. This narrowing was due chiefly to atrophy of smooth muscle, so that the elastic lamellae were crowded together with no intervening tissue except thin strands of fibrous tissue. Where this condensation was marked the individual elastic layers were barely discernible. In addition they frequently were ruptured and fragmented. The intima at the edges of the cuff was raised and thickened by a moundlike eminence of fibrous tissue, especially when 1.0 cm. cuffs had been used. This peripheral fibrous overgrowth of the intima was very scanty or completely absent when 0.3 cm. cuffs were employed. Within the field of the cuff the intima was as delicate as in the normal vessel. The fine corrugations seen grossly within 1.0 cm. cuffs were due to irregularities in the media of the vessel.

The changes observed in the vessel wall were always of the same type and approximately of the same extent, varying only in the latter respect with the size of the cuff used. Analogous alterations were found in the banded arteries of rabbits fed cholesterol.

Relation of Intimal Deposits of Lipids to the Sites of the Silver Cuffs

The amount of lipids found in the intimas of the arteries as a whole varied from none at all to numerous, heavy, almost confluent deposits. Similar unaccountable variations in lipids deposited in the femoral arteries of rabbits were observed by Harrison.[15] In general the femoral arteries had greater deposits than the carotids. It was at once apparent that when lipids were present at all they tended to accumulate in greatest amount in the vicinity of the cuffs. In some instances, when such deposits were scanty, the entire amount was limited to the regions of the cuff. When they were heavy, lipidic plaques were scattered throughout the vessel but were largest and most conglomerate where the silver bands had been placed.

The fatty deposits near the larger (1.0 cm.) cuffs were heaviest at the edges where they sometimes formed transverse linear bands 1 mm. or more wide, extending around the entire circumference. Occasionally, tail-like processes extended away from the edges of the cuff into the adjacent intima. Lipids were not found in any appreciable degree within the central portions of these larger cuffed areas. This was true even when lipidic deposits were abundant everywhere else in the vessel.

Lipids tended to overrun the entire zone of the smaller (0.3 cm.) cuffs, forming irregular plaques or complete annular bands, not limited merely to the edges. Often there was a clear zone up to 1.5 cm. in width on either side of the cuff but beyond this lipid-free area smaller, independent, spherical or oval collections of lipids were scattered at random. When two 0.3 cm. cuffs were situated within 1.0 cm. of each other on a carotid artery, no lipids whatever were found in the intervening zone even if the sites of both cuffs were heavily laden. Under similar circumstances in the femoral arteries the intervening zone often had large accumulations of lipids. This may be attributed to the fact that the more extensive periarterial fibrosis in the case of the latter artery tended to immobilize the entire area.

Histologically, the smaller lipidic aggregates consisted of clusters of spherical, swollen macrophages with foamy cytoplasm, with no other cells participating and without connective tissue proliferation. The thickening of the intima seemed to be due largely to the presence of these cells, although occasionally they were impacted against areas of fibrous tissue proliferation similar to those seen at the edges of 1.0

cm. cuffs in control animals which had not ingested cholesterol. With Sudan stains on frozen sections the bulk of the lipids was found within these phagocytes but there was always a small amount in fine-droplet form free in the intima. Crystals of free cholesterol were not seen. The entire lipidic mass appeared to be anisotropic in polarized light.

The larger collections of lipids appeared as loosely woven, pale-staining masses in sections stained by hematoxylin and eosin. There were scattered nuclei in them and a few delicate, pink-staining fibrils. Some of these nuclei were pyknotic; most of them appeared to be the nuclei of large mononuclear cells. The cell boundaries of foam cells were less readily distinguishable. In fat stains the lipids extended from the intact endothelial surface to the media and where the elastic fibers were ruptured some had found their way into this coat as well. A higher percentage of the lipids in these larger aggregates was extracellular. In sections stained for connective tissue and elastic tissue there was very little evidence of fibril formation in the plaques and no proliferation of elastic tissue filaments. For this reason the fibrous mounds which had formed at the edges of the larger cuffs, similar to those observed in control animals, were readily distinguishable. These mounds consisted of dense masses of collagen and contained fine elastic fibrils. Often the lipidic masses were piled up externally and laterally to these mounds as though they presented impermeable barriers to the passage of lipids toward the actual sites of the cuffs.

The most striking observation, however, was the finding that the cuffs in themselves did not influence the total amount of lipids deposited in any one artery. In some instances no lipids were found at any point in the artery even though the injury produced by the cuffs was just as marked as in others and even though the other arteries in the same rabbit showed the usual deposits. In other words, although the injured areas served as foci for harboring lipids, the amount of deposition was not proportional to the degree of injury, which was fairly constant in all cases; but varied rather with the amount of lipids deposited in the vessel as a whole. In Table I an attempt is made to demonstrate this point by summarizing the findings. All the data from both sizes of cuffs are grouped together and where a single artery was enclosed in more than one cuff the average amount per cuff is recorded. Three carotid and four femoral arteries had no detectable lipids at any point. There were three carotid arteries in which intimal lipids were not found in the mobile parts of the vessel but which contained slight to moderate amounts in the cuffed areas. On the other hand, all five carotid and four femoral arteries which had numerous plaques throughout the mobile portions had very extensive deposits in the regions of the cuffs.

Discussion

These results do not support the contention that lipids accumulate in the intima at points of relative immobilization because structural changes attendant on fixation may lead to increased local permeability or special affinity for lipid. Conditions favorable to cholesterol deposition were present in all nine rabbits and such deposition did occur somewhere in the arterial system in every instance. Yet in 7 of 36 arteries no lipids penetrated at sites which were invested by silver bands. In the other 29 arteries these banded areas harbored more

Table I

Relation of Intimal Deposition of Lipids at Sites of Silver Cuffs to Amount of Lipids in Other Parts of the Same Artery

	Lipids in non-cuffed areas	Lipids in and adjacent to cuffed areas					Number of arteries
		None	Slight	Moderate	Marked	Very marked	
Carotid artery	None	3	2	1	—	—	6
	Slight	—	2	1	—	—	3
	Moderate	—	—	—	2	2	4
	Marked	—	—	—	—	5	5
Femoral artery	None	4	—	—	—	—	4
	Slight	—	—	—	3	2	5
	Moderate	—	—	—	1	4	5
	Marked	—	—	—	—	4	4
	Totals	7	4	2	6	17	36

lipids than the neighboring portions of the vessels. The morphological changes in the media and externa of the vessels were not appreciably different in these two groups. If some special type of injury alone is the deciding factor in controlling deposition of lipids, then its nature is not apparent histologically.

On the other hand, the findings do favor, without furnishing direct proof, the hypothesis that relatively fixed areas of the artery serve as traps which collect both intracellular and extracellular lipidic droplets as they drift through tissue spaces of the intima. The factor which determined the amount of lipids found at the sites of the silver cuffs appeared to be the amount found in the vessel as a whole. That the localization of lipids in the cuffed regions was not purely fortuitous is indicated by the fact that more lipids were invariably located here than at any other one point in the same vessel. Other findings which favor this interpretation are the localization of lipidic masses external to, but in immediate contact with, the zones of fibrous intimal proliferation caused by the cuffs themselves, and the occurrence of lipid-free zones in the intima on either side of regions which were immobilized by the cuffs. This latter finding suggests that lipids in the immediate vicinity had drained to the banded areas of the vessel.

If it could be proved that the formation of circumscribed masses of

lipids in the intima is the result of aggregation of a finely scattered deposit due to shifting of the material within potential intimal spaces, a number of apparently unrelated and even contradictory observations on the pathogenesis of human lesions could be coördinated into a single thesis. For example, it would be possible to account for the lipidic intimal streaks unassociated with fibrous proliferation as an early phase in the formation of discrete plaques of more complex structure. These have frequently been interpreted as having an entirely independent origin from so-called true atheromatous plaques.[1]

It would also be possible to postulate that diffuse intimal proliferation is not necessarily the inevitable result of ageing but is rather due to the repeated passage of lipids through intimal tissue. In rats which were allowed to live their entire life span of about 3 years, no equivalent thickening of the intima comparable to that of man was observed.[16] The aortic media of these animals underwent changes analogous to those seen in aged human aortas. It became thicker and the elastic lamellae lost their undulant character. The value of such a comparison is limited by the marked discrepancy in life span of the two species, but it is worthy of note that intimal deposits of lipids were never observed in the arteries of senile rats.

More important, the proof of intramural convergence of lipids to fixed areas would obviate the present necessity of assigning a dual origin for the formation of these lesions as does the "combination theory" advocated by Anitschkow.[6] On the one hand it is necessary to assume that local alterations precede and prepare the site for lipidic infiltration and on the other that changes occur in the circulating blood which permit the escape of cholesterol esters and other lipids into the arterial wall. It has been exactly on the importance of the relative rôles of these two processes that much controversy has developed in recent years.

By applying the theory of movement of lipids advanced in this report, it is possible to account for most of the vagaries in distribution of the early and late aortic lesions and of those seen in young and old individuals. In infancy and childhood, all parts of the aorta being equally and quite freely movable and scarred areas being absent, there is little tendency for lipids to pool in definite spots and when present they appear as irregular, often diffuse streaks in the proximal portions of the vessel. Only at valvular attachments or at the closed aperture of the ductus arteriosus do such migrating masses come to rest. Later in early adult life when the posterior thoracic and lower abdominal portions of the vessel already exhibit a measurable reduction in postmortem elasticity[9] and anchorage to the adjacent bony structures is more rigid, such lipidic streaks tend to converge on these areas and to

form more permanent plaques with secondary changes in the tissues about them. With advancing age the movement of lipids is impeded further by plaques which have already formed and become invested by hyalinized connective tissue, and by the loss of elasticity and mobility in the remaining portions of the vessel. Eventually, if lipids continue to be deposited, the original pattern of selective localization is largely lost and the plaques are scattered more or less at random. Intercurrent syphilitic aortitis will cause many points of intimal fusion and hyalinization as well as diminished elasticity in the proximal part of the aorta and thus dislocate the movement of lipids.

Summary

Portions of femoral and carotid arteries of nine rabbits were rendered relatively immobile by being invested *in situ* by cylindrical silver cuffs of two uniform sizes. These animals were each fed 1 gm. of cholesterol daily. The changes induced in the arterial walls and adjacent tissue by cuffs alone were studied in four other rabbits. These proved to be fairly constant in type and extent. Briefly, they consisted of adventitial thickening and fibrosis, medial thinning due to atrophy of smooth muscle and condensation and fragmentation of elastic fibers. The intima remained thin and delicate save at the edges of the larger (1.0 cm. wide) cuffs where small mounds of fibrous tissue formed.

Cholesterol feeding usually led to selective localization of lipids in the intima of the arteries at the region of the cuffs. The amount of lipids present could not be correlated with the degree of injury produced by the cuffs. In 7 out of 36 arteries no lipids were found in the vicinity of the cuffs even though the injury to the vessel walls was as extensive as in the 29 others in which lipids in variable amounts were found.

The amount of lipids found at the site of the cuffs invariably exceeded that found at any other point in the vessel, indicating that conditions at these areas were favorable for their accumulation. The amount of lipids at the sites of the cuffs was directly proportional to the amount in the vessel as a whole.

These findings are interpreted as indicating that the injury itself was not the decisive factor in the deposition of lipids in the immobilized areas. Another and more plausible explanation is advanced; namely, that the immobilized areas served as foci at which the movement of lipids in intimal spaces, impelled by pulsations of the vessel wall, was arrested. The hypothesis is advanced that the points at which lipids accumulate permanently under natural conditions in arterial walls are not necessarily the same points at which they enter the intima but rather that they represent areas in which lipids are aggregated by a process of intramural migration.

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DESCRIPTION OF PLATES

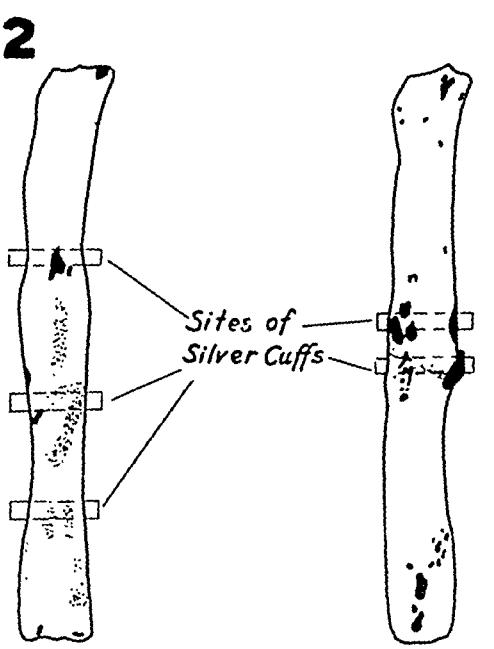
PLATE 11

FIGS. 1 and 2. A photograph and drawing of two carotid arteries showing the distribution of intimal lipids in relation to the sites of the silver cuffs. The artery on the left shows very little lipid and most of it is in the vicinity of the cuffs. The one on the right shows more extensive deposits, most of which are in the immobilized area. Adjacent to this area there is relatively little lipidic deposit but at a distance the plaques are again numerous.

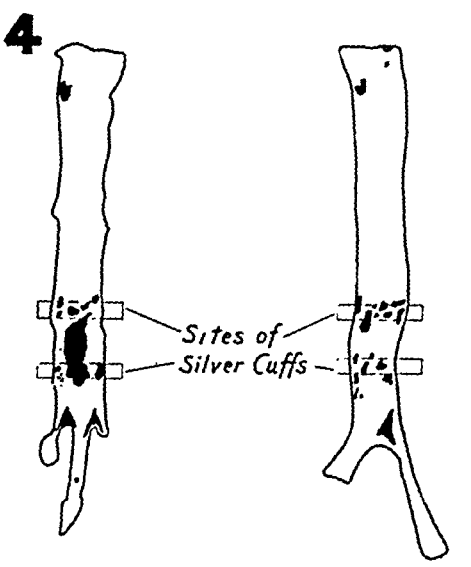
FIGS. 3 and 4. A photograph and drawing showing intimal lipids in two femoral arteries. The bulk of lipidic deposit is contained in and between the sites of the silver cuffs.

FIG. 5. Preparation stained by Weigert's and van Gieson's stains showing entire span occupied by a silver cuff, 1.0 cm. wide, on femoral artery. The adventitial fibrous tissue has been dissected away except at the edges where it enclosed the cuff. The media has been greatly thinned out and its elastic lamellae pushed together. The intima in the field of the cuff is thin but at its edges there are two moundlike elevations in which an abundance of lipid was easily demonstrable. × 8.


1


2
Sites of
Silver Cuffs


3


4
Sites of
Silver Cuffs

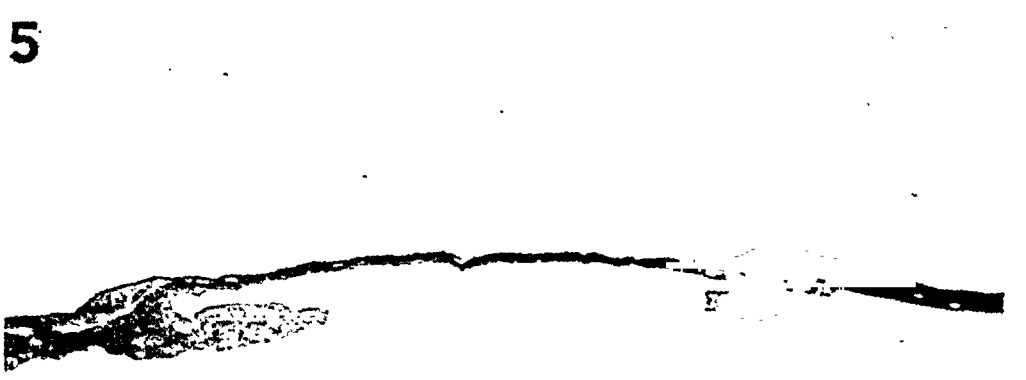

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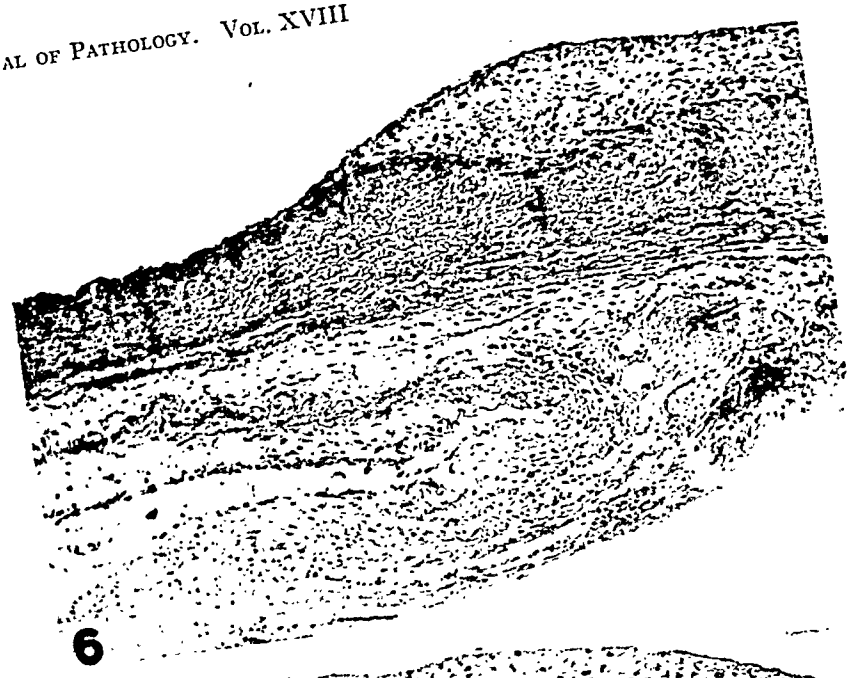
PLATE 12

FIG. 6. Section of a carotid artery stained by hematoxylin and eosin, showing a cellular and partly fibrous intimal plaque situated at the edge of a cuffed area and extending beyond it. Lipids were demonstrated in this plaque by staining frozen sections with Sudan stain. The exact position of the edge of the silver cuff is indicated in the fibrous adventitia by the horizontal slit in which the cuff was lodged. × 79.

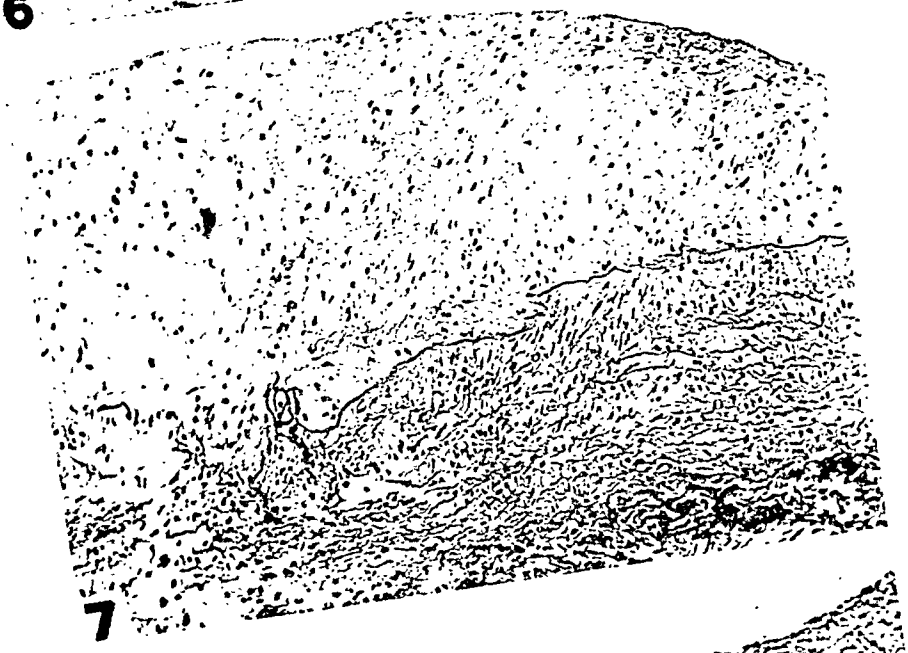
FIG. 7. A higher magnification of an intimal plaque situated over the edge of a silver cuff. The pale-staining and loosely woven texture of the plaque is due to the presence of extracellular lipidic droplets. Foam cells are also present but do not show clearly. The media at the edge of the cuff is markedly disrupted. × 103.

FIG. 8. Section of a femoral artery, prepared with Weigert's and van Gieson's stains, at the edge of a silver cuff, 1.0 cm. wide. The media is sharply narrowed at this point and over it is a crescent-shaped mound of connective tissue in the intima, containing many delicate elastic fibrils. Similar areas of intimal hyperplasia were observed in this region in rabbits which were not fed cholesterol. Just external to this intimal mound and piled up against one side of it is the edge of a lipid-containing atheromatous plaque. × 106.

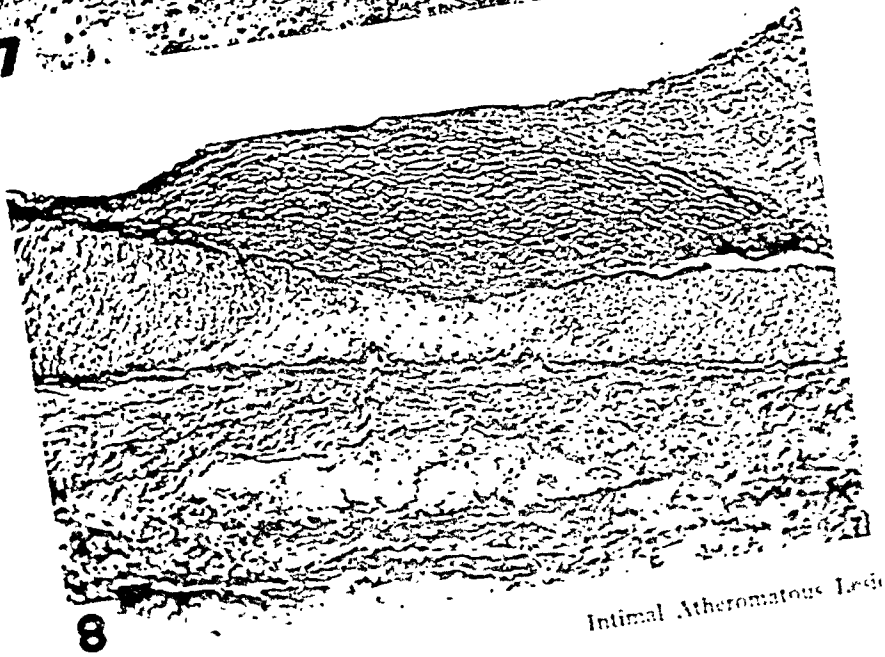
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8

Intimal Atheromatous Lesions in Rabbits

THE PATHOLOGY OF CHASTEK PARALYSIS IN FOXES *

A COUNTERPART OF WERNICKE'S HEMORRHAGIC POLIOENCEPHALITIS OF MAN

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A specific dietary disease of silver foxes, which we have recognized since 1932 as one of the important problems of the fur industry, is characterized pathologically by lesions similar to those of Wernicke's disease of man. The purpose of this paper is to describe the pathologic picture of the disease as it occurs in foxes and to clarify the relationship it bears to Wernicke's disease. The epidemiologic and nutritional aspects are described in greater detail elsewhere.[1]

We first observed this disease among foxes in February, 1932, when it appeared among the animals on the Chastek fur farm in Minnesota, and, during a period of 1 month, killed one-third of the entire ranch population of more than 200 foxes. In 1936, one of us (Green [2]) described the disease as a clinical entity and pointed out its occurrence as related to the feeding of fresh fish. A year later we [3,4] reported its experimental reproduction and the cause as a vitamin B deficiency. Carlström and Jonsson [5] reported the disease in Sweden in 1938 and obtained recovery of one fox by the injection of pure vitamin B_1. More recently, Ender and Helgebostad [6] have confirmed our reproduction of this disease experimentally, describing the condition as experimental beriberi. We have studied more than ten outbreaks in five states since 1932 and have shown that the natural outbreaks of the disease are usually induced by feeding foxes and minks fresh fish and that the disease is purely a vitamin B_1 deficiency. In a recent experiment a group of 12 foxes given a diet including carp as 20 per cent of the ration developed Chastek paralysis within 3 weeks, while a group of 16 foxes simultaneously receiving an identical ration plus 25 mg. of thiamin hydrochloride a day remained entirely well. The mechanism by which the presence of fish in the ration brings about deficiency of vitamin B_1 is not clear, but synthetic thiamin hydrochloride in large doses will prevent Chastek paralysis.

* From the coöperative investigations of the University of Minnesota, the Minnesota Department of Conservation, and the Fish and Wildlife Service, U. S. Department of the Interior, with the assistance of Fromm Laboratories, Grafton, Wis.

Presented at the Forty-First Annual Meeting of the American Association of Pathologists and Bacteriologists, New York City, April 10, 1941.

Received for publication, May 8, 1941.

The usual history in a typical outbreak is as follows: Fish is introduced sometime in the fall or winter as from 10 to 50 per cent of the total ration. No untoward results are observed for a period of from 3 to 6 weeks. The foxes then go "off feed" and after a week or 10 days, individual animals have an abnormal gait; they may stagger or simply appear to be weak. Within 24 to 48 hours, such animals are usually seriously ill. Severe ataxia ensues, accompanied by a progressive spasticity which becomes so severe that finally the animals are unable to stand. Hyperesthesia may be marked, and the foxes usually moan constantly, as though in great pain, during the last 12 to 24 hours of life. The course of the disease is progressive; convulsions may occur, and animals usually die within 24 hours after developing ataxia and spasticity.

New cases appear in rapid succession. As already mentioned, we have observed the decimation of one-third of a ranch population of more than 200 foxes in a period of 1 month. Dramatic cessation of the outbreak can be brought about by removing fish from the ration and feeding a diet containing large proportions of liver. Animals in an early stage of the disease, or even those that show definite ataxia and early spasticity, can be cured by injecting thiamin parenterally and tube-feeding water, milk and liver juice.

Pathologic Findings in Organs Other Than the Brain

Although the most significant and diagnostic lesions of this disease are limited to the brain, many characteristic changes are found in other organs. The latter will be discussed first.

The nutrition of animals usually appears, at necropsy, to have been normal. This is somewhat surprising in view of the period of inappetence which precedes the onset of neurologic symptoms. Perhaps the small amount of food eaten by the foxes during this period is sufficient to keep them in good nutrition inasmuch as they are relatively inactive and maintain a low metabolic rate while sick with Chastek paralysis, as shown by subnormal body temperatures.

The lungs are negative except in an occasional animal in which there is a slight bronchopneumonia. The pleural, pericardial and peritoneal surfaces are normal. As a rule, no food is found in the stomach or intestines. Ulcers of the gastric mucosa are present more often than in foxes that die from other diseases. In different groups of foxes with Chastek paralysis, ulcers have been found in from 10 to 30 per cent of the animals. There is often a considerable amount of bile-covered mucus throughout the gastro-intestinal tract. Occasionally red blood, or black, tarry material is present in the intestines.

Degeneration of the liver is one of the most constant features of this

disease. The gross appearance of this organ, however, is variable, as it may be either obviously fatty or of a dark mahogany color. In either case the liver is usually soft and friable. Occasionally it has ruptured before death, causing hemorrhage into the peritoneal cavity. A moderate degree of icterus is seen in from 10 to 20 per cent of the foxes. In most animals the gallbladder is distended with bile. In about 5 per cent the liver is normal grossly and upon microscopic section is found to show only a small amount of fatty metamorphosis. Microscopic study of livers that were either fatty or of a mahogany color at necropsy shows a severe degree of lipoidosis to be present. In addition there may be necrosis of hepatic cord cells, as well as congestion and hemorrhage.

The spleen almost invariably is normal at necropsy, and microscopic sections show no change other than a moderate degree of hyperplasia of the lymph follicles. The kidneys are generally pale, and microscopically are found to have a few foci of lymphocytic infiltration among the tubules. There may also be some tubular degeneration. The urinary bladder, as a rule, is normal and may be empty or may contain a moderate amount of urine. The thyroid, adrenal, pancreas and salivary glands are usually normal.

Grossly, the heart may show cloudy swelling, and hemorrhages are present in about one-half the animals. Microscopically, the myocardium is sometimes normal but more often there are a few hemorrhages. Occasionally extensive pathologic alterations are found. The nucleoli of the myocardial cells may be so greatly enlarged as to resemble, superficially, intranuclear inclusions of a virus disease (Fig. 5). Areas of degeneration of myocardial fibers are seen associated with the hemorrhages. Proliferation of connective tissue, which extends outward from the perivascular areas to form a fairly dense meshwork among the degenerated cardiac muscle fibers, is also occasionally seen. The myocardial fibers in some cases appear to have a moderate intracellular edema, as evidenced by an increase in the size of the axial space. The combination of large nucleoli, focal necrosis of fibers, proliferation of connective tissue arising from the vascular tree and hemorrhages is diagnostic of Chastek paralysis, but one or more of these changes are lacking in a large proportion of cases. It appears that the neurologic manifestations of the disease usually kill the fox before cardiac lesions develop sufficiently to be diagnostic.

Pathologic Findings in the Brain

In the brain, the characteristic lesions of Wernicke's disease in man and of Chastek paralysis, which we believe to be the counterpart of that disease in foxes, are definitely focal in character and are highly characteristic in distribution and appearance. In both diseases strik-

ing proliferation of the endothelium and of the adventitia of small vessels occurs in focal areas. Irregular dilatation or varicose deformity of vessels is characteristic of both diseases. Numerous, small, parenchymal hemorrhages result from the degenerative vascular changes. A fully developed lesion appears as a hyperemic area in which there is an apparent marked increase in the number of small vessels, all of which are very cellular, and many of which are irregularly shaped (Figs. 2, 3 and 4). Alexander [7] demonstrated that the reported increase of vessels in the lesions of Wernicke's disease is only apparent and that with appropriate technics it may be shown that varicose dilatation and endothelial and adventitial proliferation have caused vessels that are not usually seen to become easily visible. Presumably the same is true in Chastek paralysis.

Degenerative parenchymatous changes occur in Chastek paralysis but are not so specific as the vascular changes. Nerve cells in the midst of a typical vascular lesion usually show degeneration or disappear altogether. As a rule, some proliferation of neuroglial elements is apparent. In an occasional animal, lesions are found in which fat-granule cells are exceedingly numerous, but more often these cells are relatively few. It is clear that the vascular changes do not represent a secondary phenomenon of the sort that follows ordinary tissue damage.

Although the histologic characteristics and general distribution of vascular lesions in the brains of foxes with Chastek paralysis have been familiar to us and have served as a basis for routine diagnosis during the past 5 years, we have only recently attempted to define precisely the location of these lesions, in order to make accurate comparisons with Wernicke's disease.

The sections used in studying the distribution of these vascular lesions were drawn from our regular files of animals submitted primarily for diagnosis. Our routine procedure in establishing a diagnosis includes obtaining eight or ten sections of the central nervous system from the following regions: cervical cord, medulla oblongata, mesencephalon, basal ganglia, cerebellum, and the olfactory, frontal, occipital and temporal lobes of the cerebrum. The exact location from which these sections are taken varies considerably in different animals.

Sections from 25 foxes were surveyed and more than 50 preparations were selected as showing typical lesions of Chastek paralysis. Of these foxes, 13 had died in ranch outbreaks and 12 had succumbed to experimentally-induced Chastek paralysis. These sections, in which well developed lesions were found, included 21 cut through the medulla, 12 through the cerebellum, 13 through the mesencephalon, 6 through the basal ganglia and 6 through the cerebral cortex. From these we have

obtained a composite picture of the pathology of Chastek paralysis. There are parts of the brain which are still unexplored, but the principal facts concerning the anatomic distribution of the characteristic vascular lesions are now available from the studies that have been completed.*

Wernicke,[8] in his original paper, commented on the fact that the lesions in human patients presented a bilateral symmetry with almost mathematical exactitude. No less striking is this symmetrical pairing of lesions in foxes with Chastek paralysis. Even before the microscopic pathology of this disease had been studied, we considered the gross appearance of bilaterally symmetrical hemorrhages in the brain stem highly characteristic of Chastek paralysis and quite different from the irregular distribution of the hemorrhages in other diseases, such as epizootic fox encephalitis. More recently it has become evident that the symmetry is also manifested by histologic changes. If the dorsal nucleus of the vagus nerve is involved on one side of the medulla, the corresponding nucleus on the opposite side will be involved by a lesion.

Another striking feature is that these lesions develop only in the gray substance of the brain. Never have we found a lesion in the midst of the pyramids or of the other portions of the brain which are devoid of nerve cells. This is an interesting fact when we remember that histologically the principal changes appear to be vascular.

The characteristic lesions of Chastek paralysis are limited not only to the gray substance of the brain but also, as a rule, to certain specific nerve centers. The term, paraventricular nuclei, has been used to describe, in a general way, the distribution of lesions in Wernicke's disease. This term applies equally well to Chastek paralysis in so far as the lesions in the dorsal nucleus of the vagus, the vestibular nuclei, the inferior colliculi, the nucleus of the third cranial nerve and the walls of the third ventricle are concerned. Typical lesions are also commonly found in the inferior olive and in the cortex of the splenial gyrus, both of which are a considerable distance from the ventricular system. On the other hand, lesions are often absent from the superior colliculi and the nucleus of the fourth cranial nerve, two structures which are well within the paraventricular gray matter.

There are certain nuclei that are practically never involved and others that show vascular lesions regularly. The presence or absence of lesions cannot be correlated solely with proximity to the ventricular system.

* M. A. Schadewald, formerly of the University of Minnesota and now at the University of West Virginia, identified, or verified the identity of, the nerve centers mentioned in this paper.

Distribution of the Characteristic Vascular Lesions in the Medulla Oblongata

In the medulla oblongata a lesion is usually present in the dorsal nucleus of the vagus. If it is severe, it may extend over the nucleus of the hypoglossal nerve, but the latter nucleus is seldom involved independently of the nucleus of the vagus. Typical lesions are frequently present in the medial vestibular nucleus, and often extend into the lateral, the superior, or the descending vestibular nuclei. It is not uncommon to see a marked vascular lesion in the medial vestibular nucleus, while one or more of the other vestibular nuclei are entirely normal. A lesion with very severe vascular changes is sometimes seen among the nerve cells lateral to the area ovalis of the corpus restiform and adjacent to the floor of the fourth ventricle.

Of four animals from which the selected sections passed through the upper end of the nucleus cuneatus and nucleus gracilis, three showed typical vascular lesions. Hemorrhages are common in the reticular formation but proliferative lesions do not occur there. In all cases, the pyramids were negative. Of six animals in which the sections included the inferior olive, three showed typical lesions. This is noteworthy, as already mentioned, in that it constitutes an exception to the general rule that lesions of this disease occur in the paraventricular area.

The bilateral symmetry of the lesions of Chastek paralysis is obvious in the medulla as well as in other parts of the brain. The few exceptions may be attributed to differences in degree of severity of lesions on the two sides. Occasionally, for example, lesions located primarily in the dorsal nuclei of the vagus may extend into the nucleus of the hypoglossal nerve on one side of the medulla but not on the other. It should be emphasized, however, that marked differences in severity of involvement on the two sides of the brain are the exception; it is the rule to find paired lesions of equal severity.

Distribution of the Characteristic Vascular Lesions in the Cerebellum

When present in the cerebellum, lesions are almost invariably located in folia close to the fourth ventricle. Apparently the vascular changes spread from the surface inward through the granular layer and into the molecular layer. Proliferation and irregular dilatation of vessels are most prominent in the granular layer, but hemorrhage is more extensive in the molecular layer and may force its way well into the white substance. Here, as elsewhere, the vascular pathology is found in the gray substance with only an occasional abnormal vessel extending into the adjacent medullary substance.

Distribution of the Characteristic Vascular Lesions in the Mesencephalon

The quadrigeminal plate is frequently involved and in all such cases lesions are present in the inferior colliculi. Occasionally the vascular changes extend anteriorly so as to form lesions in the superior colliculi, but more often sections through the superior colliculi are normal.

Ventral to the aqueduct, the nucleus of the third cranial nerve is the most common location of typical vascular lesions. The nucleus of the fourth nerve is seldom involved.

We have nearly always found the nucleus rubra to be normal, and have never seen a well developed lesion in the substantia nigra or basis pedunculi.

Distribution of the Characteristic Vascular Lesions in the Diencephalon

Many sections through the diencephalon of the selected 25 foxes were negative but some showed lesions in the thalamus and in the habenular nuclei. These lesions in some animals did not involve the parts closest to the third ventricle but were separated from the ventricular surface by a narrow zone of normal brain tissue.

Distribution of the Characteristic Vascular Lesions in the Cerebral Cortex

As a rule, routine sections from the olfactory, frontal, occipital and temporal lobes of the cerebrum are negative; but in a few of the foxes given special study, well marked vascular lesions associated with hemorrhages were present in the frontal and occipital lobes. These lesions were found most often in the vicinity of the splenial fissure, both in the frontal lobe near the anterior end of the fissure and in the occipital lobe close to its posterior end. In the occipital lobe there was, in some cases, bilateral selective involvement of the brain substance dorsal and posterior to the splenial fissure; *i.e.*, in the gyrus splenialis, the gyrus fornicatus on the opposite side of the fissure being normal or showing only moderate congestion. In the frontal lobe, the lesions were found on both sides of the splenial fissure. Many animals showed only congestion, with or without a mild endothelial proliferation, and some showed no lesions at all in this region. In an occasional animal, typical vascular lesions were found in foci adjacent to the fissura ectolateralis, the fissura suprasylvia posterior and the sulcus suprasylvius posterior.

In a few instances foci of varicose deformity and proliferation of small vessels involved the meninges as well as the subjacent cerebral substance. In all lesions of the cortex, the most severe vascular changes have been found in the superficial layers: *i.e.*, the molecular and outer

granular layers. Hemorrhages in many cases extended beyond the site of vascular damage, but the foci of degenerated and proliferated vessels were not found outside of the gray substance.

Discussion

A comparison of the disease which we designate as Chastek paralysis with that which Wernicke [8] described in 1881 as "die acute, hämorrhagische Poliencephalitis superior" shows some striking similarities.

Clinically, ataxia was noted by Wernicke, who stated: "Der Gang der Kranken wird taumelnd und zeigt eine Combination von Steifheit mit Ataxie, die am meisten an die Ataxie der Alcoholiker erinnert." [The walk of the patient becomes reeling and shows a combination of *stiffness with ataxia* (italics ours), which reminds one most strongly of the ataxia of alcoholic persons.] Progressive ataxia and spasticity are outstanding clinical features of Chastek paralysis.

Limitation of eye movements, noted by Wernicke, has not been studied in foxes, but there is ample basis in the pathology of the disease for some disturbance of ocular movements.

Principal emphasis was placed by Wernicke on the characteristic distribution of hemorrhagic lesions, as seen at autopsy. In summarizing briefly the findings in his second patient, he stated: "Am Gehirn derselbe Befund, wie im vorigen Falle. Die Blutungen gehen nirgends über die punktförmige Beschaffenheit hinaus und sind auf das centrale Höhlengrau des dritten und vierten Ventrikels sowie des Aquaeductus beschränkt." [The findings in the second patient were the same as in the first patient. The hemorrhages were confined to the central gray matter of the third and fourth ventricles and the aqueduct.] This statement and that already mentioned, "Die Veränderungen sind beiderseits mit fast mathematischer Genauigkeit gleich" [the lesions are equal with almost mathematical exactitude on the two sides of the brain], would both be valid in a general description of the characteristic lesions of Chastek paralysis of foxes (Fig. 1).

The reports of microscopic studies that Wernicke included in his original description were rather meager. However, they did point out that the hemorrhages and associated tissue reactions were confined to the nerve centers and "erstreckten sie sich nirgends auf die dem grauen Boden benachbarten Gebilde und Fasermassen" [never extended into the structures and nerve fiber masses in the vicinity of the gray matter]. This is a highly characteristic feature of Chastek paralysis, also. Never have we found a typical proliferative vascular lesion which developed outside of the gray matter.

In one case Wernicke mentioned that the region of the habenulae was

involved on both sides. This is a fairly common site of bilateral lesions in Chastek paralysis.

In his three cases, Wernicke did not find any marked changes in the vessel walls, nor did he observe any striking degree of endothelial proliferation. Subsequent workers have contributed much toward a more complete description of the vascular pathology in Wernicke's disease, and their findings coincide very closely with our observations in Chastek paralysis.

Focal lesions in which irregular dilatation of vessels and proliferation of endothelial and adventitial elements give the appearance of a marked increase in the number of small vessels, are known to characterize Wernicke's disease. These changes are identical with those of Chastek paralysis. In both diseases, hemorrhage is usually present in the midst of the vascular lesions.

Alexander, Pijoan and Myerson [9] have obtained evidence from their studies of human cases and their experimental observations on pigeons, as well as from the observations of Zimmerman [10] on dogs, of Prickett [11] on rats and of Pappenheimer and Goettsch [12] on chicks, that in these various species there can be produced a disease with distinctive pathologic features entirely analogous to those of Wernicke's disease in man. The combined evidence strongly supports Alexander's contention that Wernicke's disease of man and the parallel disorders of lower animals are due to a deficiency of vitamin B_1 existing in the presence of an adequate supply of other vitamins.

Chastek paralysis clearly belongs to the same group of diseases. It is the only one of them that occurs as a spontaneous disease in large animal populations. Dietary studies reported elsewhere would seem to prove independently of the pathologic evidence that Chastek paralysis is a B_1 avitaminosis.

A survey of clinical reports of patients with Wernicke's disease suggests that a disturbance of gastric function, perhaps achlorhydria, may be an important factor in that disease. One of the patients described in Wernicke's original report had severe gastritis as a result of sulfuric-acid poisoning; a considerable number of patients with
the stomach have developed Wernicke's disease (E
man [13]); one case in which a patient with pernicious
nicke's disease is mentioned by Alexander, Pijoan and
it is probable that many of those with chronic
from gastritis with a consequent disturbar

There can be no reasonable doubt tha
Chastek paralysis, and it seems possible
produces the B_1 avitaminosis by chemicall

foxes' stomachs. If this occurs, achlorhydria may be a factor of considerable importance, inasmuch as thiamin is readily split in a neutral or alkaline solution but is stable in an acid medium. A study of gastric secretion in normal foxes and in foxes with Chastek paralysis is now in progress.

The selective localization of lesions of Chastek paralysis in certain nerve centers of the brain is probably of physiologic significance. It is known (Quastel [14]) that vitamin B_1 is necessary for the normal oxidation of glucose by brain tissue. In the absence of that vitamin there is an abnormal accumulation of pyruvic acid. Whether or not the lesions of Chastek paralysis and of Wernicke's disease represent a specific histologic response to an excess accumulation of pyruvic acid in the region of nerve cells affected by a lack of vitamin B_1 can be determined only by further experimentation. From the limitation of lesions to nerve centers and their selective distribution in the gray matter, it appears that some dysfunction of specific nerve cells resulting from a deficiency of vitamin B_1 is the basis for the observed vascular changes as well as for the parenchymatous degeneration.

Among nursing fox pups an acute form of Chastek paralysis, with a mortality rate of more than 30 per cent in 3 days, has been observed on two ranches. Fish or fish products constituted 16.5 per cent and 9.7 per cent of the rations on these ranches. At the time the pups were afflicted, the disease was just beginning to appear in adult animals.

Pathologically, the several adult animals that died in these outbreaks showed the usual lesions of Chastek paralysis. In the pups, however, the typical lesions were not present in the brain; no foci of vascular proliferation and varicose deformity were found, in spite of a careful search. Apparently the disease was so acute in nursing pups that death occurred before the characteristic histologic changes developed.

Summary

1. Chastek paralysis is an economically important, fatal, neurologic disease of foxes which is caused by a 10 per cent content, or more, of certain types of fresh whole fish in the ration. It can be prevented by adding large amounts of thiamin to the diet.

2. At autopsy the liver shows severe injury, and the heart occasionally contains characteristic lesions.

3. In the brain, bilaterally symmetrical lesions, limited to certain nerve centers predominantly in the paraventricular region, are diagnostic of Chastek paralysis. In these lesions there are irregular dilatation and striking proliferation of small vessels. The histologic changes and

the highly characteristic distribution of the lesions in this disease make Chastek paralysis the pathologic counterpart of Wernicke's hemorrhagic polioencephalitis of man.

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DESCRIPTION OF PLATE

PLATE 13

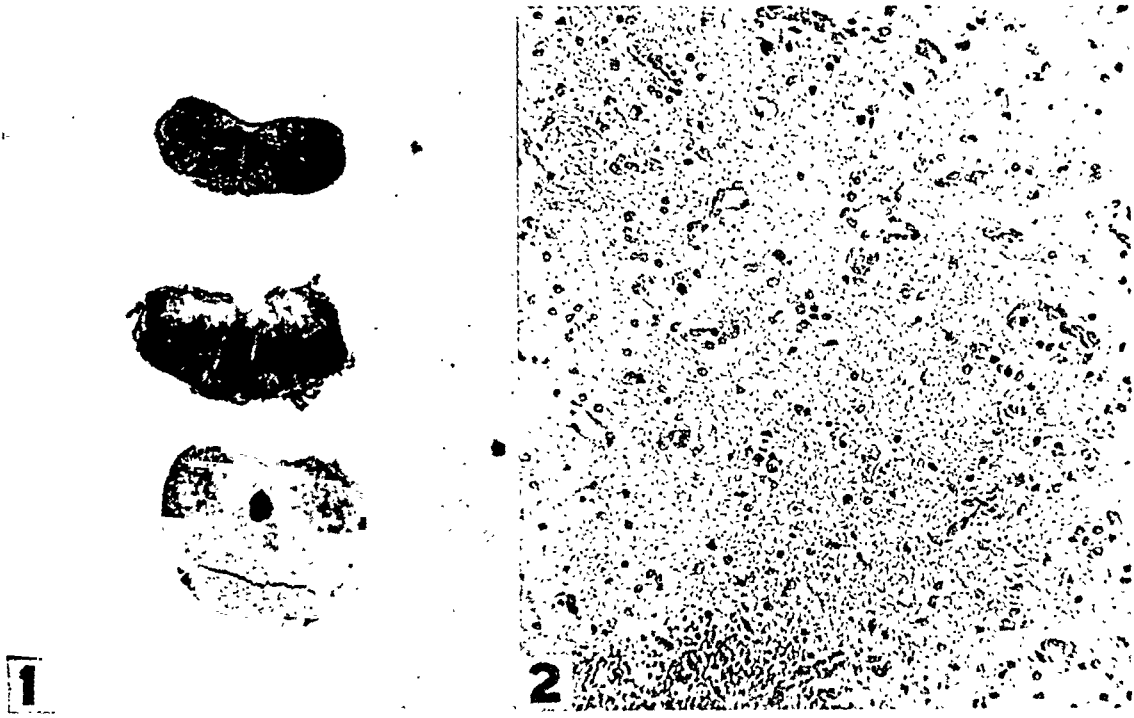
FIG. 1. Sections through the medulla oblongata and mesencephalon of three foxes showing the bilateral symmetry and specific localization of lesions in Chastek paralysis.

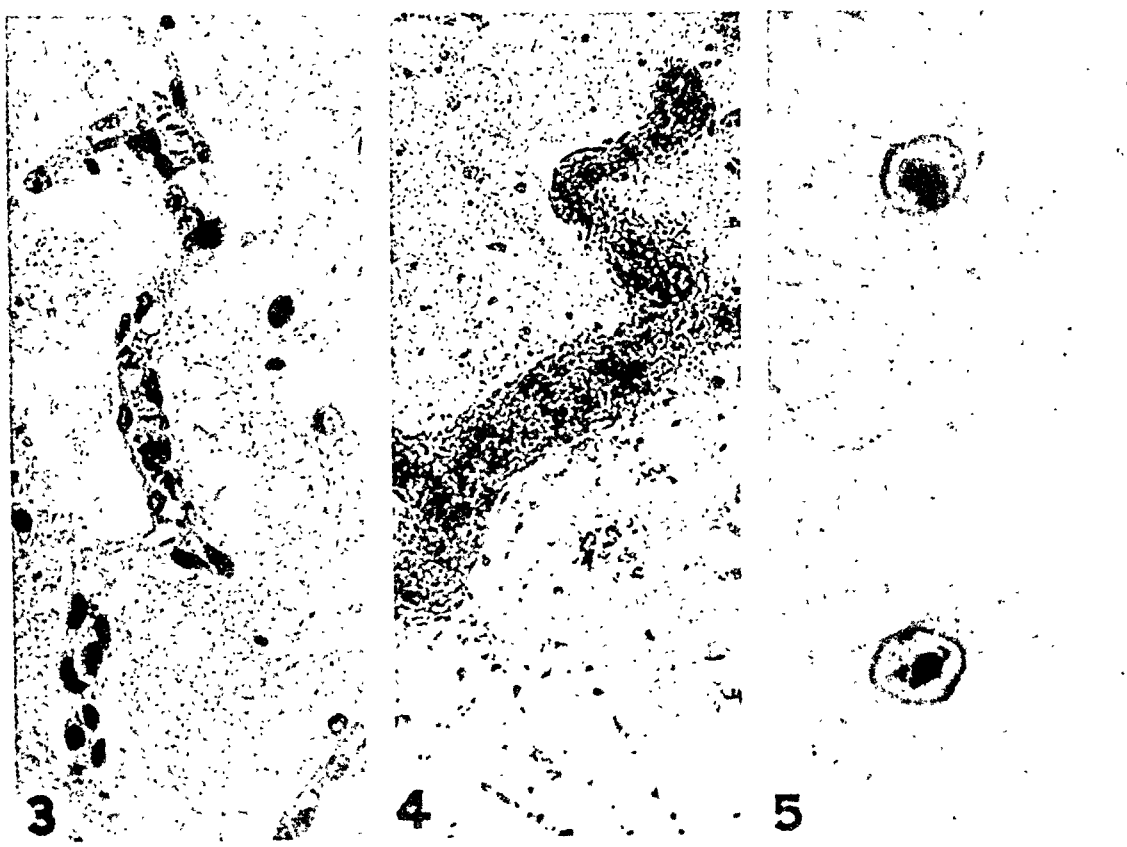
FIG. 2. Lesion in the quadrigeminal plate of fox no. R-25055. Endothelial swelling is marked in the small vessels. An area of hemorrhage is present near the lower border of the field. Hematoxylin and eosin stain. × 150.

FIG. 3. Medulla oblongata, fox no. A-899, from a ranch outbreak. Marked endothelial swelling is present in a small vessel. Hematoxylin and eosin stain. × 900.

FIG. 4. Medulla oblongata, fox no. A-897, from a ranch outbreak. Blood vessel selected to show tortuosity and irregular dilatation. Hematoxylin and eosin stain. × 200.

FIG. 5. Heart, fox no. R-25053, from a ranch outbreak. Such large nucleoli are seen in the cardiac muscle of some cases of Chastek paralysis. They have not been found in foxes which have died from other causes. Hematoxylin and eosin stain. × 2200.





Evans, Carlson and Green

INTERCAPILLARY GLOMERULOSCLEROSIS *

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In 1936, Kimmelstiel and Wilson [1] described eight instances of a renal lesion which consisted of hyaline thickening of the intercapillary connective tissue of the glomeruli, associated with arteriosclerosis. They termed the lesion "intercapillary glomerulosclerosis." Of their eight patients, seven gave a history of diabetes mellitus. Clinical data on the eighth were lacking. Anson [2] reported six additional cases in 1938. Newburger and Peters [3] tabulated the data of these reports, adding four autopsied cases of their own. According to them, the clinical syndrome associated with intercapillary glomerulosclerosis included diabetes mellitus, hypertension, widespread edema, albuminuria, hyposthenuria, nitrogen retention, hypo-albuminemia, retinal arteriosclerosis and, occasionally, heart failure and anemia. The syndrome as outlined by Porter and Walker [4] is essentially similar.

It is the purpose of this paper to point out that, although intercapillary glomerulosclerosis in its more advanced state is seen only in cases of diabetes, it is not of necessity associated with a particular clinical syndrome which includes diabetes mellitus.

The clinical histories, autopsy protocols and microscopic sections of all cases of diabetes and of arteriolar nephrosclerosis autopsied since 1909 at the Presbyterian Hospital, New York City, were reviewed. Satisfactory microscopic preparations were available from 144 cases in the former group and from 126 in the latter. Intercapillary glomerulosclerosis was noted in 33 of the 144 cases of diabetes mellitus, an incidence of 22.9 per cent (Fig. 1). Thirty-two instances, or 25.4 per cent, were found among the 126 cases of arteriolar nephrosclerosis without diabetes (Fig. 2).

The microscopic sections of the kidneys of 199 cases of glomerulonephritis autopsied during the same period were reviewed. Among these, 13 instances were found in which the lesions of many of the glomeruli were indistinguishable from those of intercapillary glomerulosclerosis (Fig. 3). Nine additional examples of this lesion were found among 81 cases of generalized arteriolar sclerosis without associated diabetes or renal disease (Fig. 4).

The incidence of intercapillary glomerulosclerosis in these v conditions is summarized in Table I.

* Received for publication, May 31, 1941.

One additional instance of early intercapillary glomerulosclerosis (autopsy no. 13545) was accidentally encountered in a female, 47 years old, with cirrhosis of the liver and Banti's syndrome who died following splenectomy. A few sclerotic arterioles were found in the kidney and pancreas of this case.

The age incidence of intercapillary glomerulosclerosis varied somewhat in the different groups. The average age at death of the patients with diabetes was 59.6 years; of those with arteriolar nephrosclerosis, 45.2 years; of those with generalized arteriolar sclerosis, 61.7 years, and of those with glomerulonephritis, 27 years. It is of interest that 82 per cent of the patients with both diabetes and intercapillary glomerulosclerosis were females, whereas only 54 per cent of the entire group of patients with diabetes were females. No such disproportion in sex distribution was noted in any of the other groups.

The pertinent clinical and pathologic data of these 88 cases of intercapillary glomerulosclerosis are summarized in Table II, according to the various categories of disease in which they were classified.

TABLE I

Incidence of Intercapillary Glomerulosclerosis

	Total no. cases	Intercapillary glomerulosclerosis	
		No. cases	Per cent
Diabetes mellitus	144	33	22.9
Arteriolar nephrosclerosis	126	32	25.4
Generalized arteriolar sclerosis (without diabetes or renal disease)	81	9	11.1
Glomerulonephritis	199	13	6.5

TABLE II

Intercapillary Glomerulosclerosis. Summary of Clinical and Pathologic Features

	Diabetes mellitus	Arteriolar nephro-sclerosis	Generalized arteriolar sclerosis	Glomerulo-nephritis	Total
Intercapillary glomerulosclerosis (number of cases)	33	32	9	13	88
	(*per cent*)	(*per cent*)	(*per cent*)	(*per cent*)	(*per cent*)
Albuminuria	83*	97	57	100	86
Hypertension (systolic pressure greater than 150 mm. Hg)	50*	94‡	67	77	71
Nitrogen retention	48†	93*	0*	92	70
Edema	30	47	22	38	36
Heart failure	42	56	22	15	41
Anemia (Hgb. less than 75%)	47*	78	0	77	59
Cardiac hypertrophy (greater than 400 gm.)	38‡	91	78	85	68
Arteriolar sclerosis, generalized	94	94	100	46	86
Arteriolar sclerosis, renal	100	100	100	100	100
Arteriosclerosis	64	88	78	31	80

* Data not recorded in 5 cases.
† Data not recorded in 12 cases.
‡ Data not recorded in 1 case.

Discussion

The results of serum protein determinations and of retinal examinations were available in too few cases to permit inclusion of these data. In our cases edema, except in glomerulonephritis, was generally dependent upon the presence of cardiac failure; and the anemia was usually secondary to renal decompensation. The clinical features which have been regarded as characteristic of intercapillary glomerulosclerosis are lacking in an appreciable proportion of these cases, among patients with diabetes as well as in the group as a whole. It is evident that the clinical signs and symptoms are not specific.

Herbut [5] and Allen [6] emphasized the specificity of the lesion of intercapillary glomerulosclerosis and suggested its use by the pathologist as a criterion for the morphological diagnosis of diabetes mellitus.* On the other hand, Kimmelstiel and Wilson,[1] in their original paper on glomerulosclerosis, called attention to the occurrence of identical lesions in the early stages of intercapillary glomerulonephritis unassociated with diabetes. Newburger and Peters [3] also studied a case of subacute glomerulonephritis without diabetes or generalized vascular disease in which the renal lesions were suggestive of intercapillary glomerulosclerosis. Our material includes 13 cases of glomerulonephritis, 10 chronic and 3 subacute, in which glomerulosclerosis was noted.

Our study indicates, further, that intercapillary glomerulosclerosis occurs not infrequently in association with conditions other than diabetes and glomerulonephritis, notably arteriolar sclerosis and arteriolar nephrosclerosis.

The case of cirrhosis of the liver, in which early lesions were seen associated only with mild arteriolar changes, is evidence that the study of routine sections of kidneys, with the possibility of glomerulosclerosis in mind, may lead to the discovery of other instances not associated with diabetes or renal disease.

It is important to note, however, that in every case where glomerulosclerosis was widespread or marked, review of the case record revealed a history of diabetes mellitus.

The frequency of arteriosclerosis and arteriolar sclerosis in patients with diabetes is well known. It has often been commented upon in association with intercapillary glomerulosclerosis as well. Nevertheless, the most striking finding emerging from our study is the extremely

* Since this paper was submitted for publication, the material upon which Dr. Allen's presentation was based has been published in the Archives of Pathology (So-called intercapillary glomerulosclerosis—a lesion associated with diabetes mellitus. Morphogenesis and significance. *Arch. Path.*, 1941, 32, 33–51.)

high incidence of arteriosclerosis and arteriolar sclerosis in these 88 cases of intercapillary glomerulosclerosis, and, in particular, the occurrence of sclerosis of renal arterioles in every instance. Newburger and Peters [3] suggested that the primary lesions were those of the arterioles, and that the diabetes and hypertension, as well as the renal changes, might be secondary.

Seventy of our cases of diabetes mellitus also showed generalized arteriolar sclerosis. The diagnosis of intercapillary glomerulosclerosis was made in 31 of these, an incidence of 44.3 per cent. Furthermore, diabetes and nephrosclerosis occurred together in 22 instances and, of these, 13, or 59.1 per cent showed the lesions of intercapillary glomerulosclerosis. Anson [2] noted the presence of nephrosclerosis in all of his six cases.

Attempts to correlate these glomerular changes with hyalinization of the islets of Langerhans and with the specific therapy of diabetes were fruitless. Of 42 of our patients with diabetes dying before the introduction of insulin, 3 cases showed the lesions of intercapillary glomerulosclerosis.

Summary

The autopsy records of 550 patients with diabetes mellitus, arteriolar nephrosclerosis, generalized arteriolar sclerosis and glomerulonephritis have been reviewed. Among them, 87 instances of intercapillary glomerulosclerosis were found. In its advanced form, this condition was always associated with diabetes mellitus, and was present in 59.1 per cent of the cases of diabetes with arteriolar nephrosclerosis. Less severe degrees of glomerulosclerosis were found with equal frequency in cases of arteriolar nephrosclerosis without diabetes, and somewhat less often in cases of generalized arteriolar sclerosis and glomerulonephritis. One additional instance was encountered in a case of cirrhosis of the liver without diabetes, renal disease or generalized vascular disease. Sclerosis of renal arterioles has been found to be invariably associated with intercapillary glomerulosclerosis.

Note: We are indebted to Dr. Walter W. Palmer and to Dr. Allen O. Whipple for permission to use the clinical records of the patients included in this study.

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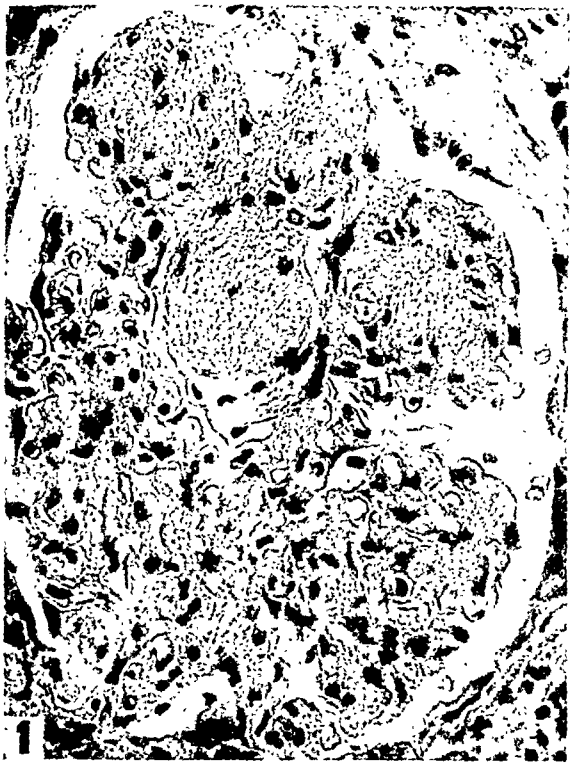
PLATE 14

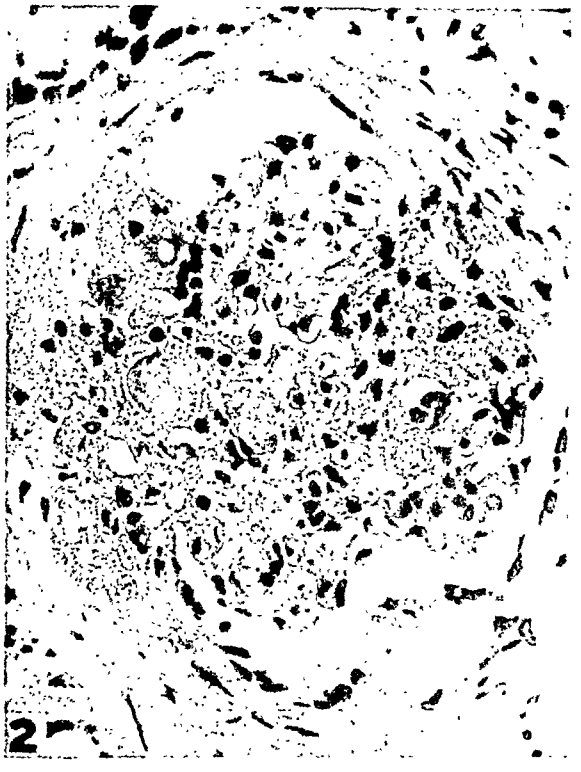
FIG. 1. Glomerulus from a case of diabetes mellitus (autopsy no. 13,286). Hematoxylin and eosin stain. × 394.

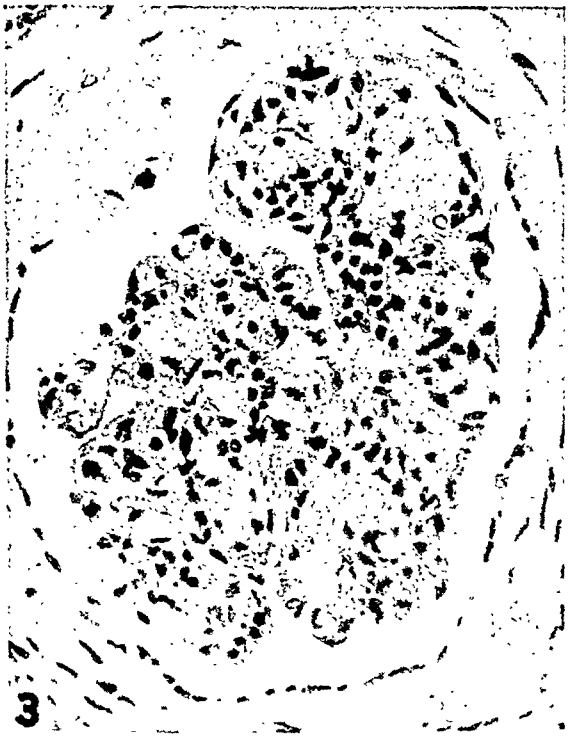
FIG. 2. Glomerulus from a case of arteriolar nephrosclerosis without associated diabetes (autopsy no. 11,757). Hematoxylin and eosin stain. × 394.

FIG. 3. Glomerulus from a case of subacute glomerulonephritis without associated diabetes (autopsy no. 11,459). Hematoxylin and eosin stain. × 394.

FIG. 4. Glomerulus from a case of generalized arteriolar sclerosis without associated diabetes or renal disease (autopsy no. 11,730). Hematoxylin and eosin stain. × 394.









Horn and Smetana

Intercapill

GLOMERULONEPHRITIS IN PARTIALLY NEPHRECTOMIZED RATS*

Relation to Administration of Sulfapyridine

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Nephritis in rats is commonly exudative in character and is caused by ascending urinary infections or by bacteremia. However, in recent years experimental glomerular lesions, resembling those of human glomerulonephritis, have been produced in rats by the administration of nephrotoxic sera [1,2] or high protein diets.[2,3] A report by Jaffe,[4] in 1929, of an isolated instance of proliferative, non-exudative glomerulonephritis in a stock rat emphasizes the rarity of this lesion as a spontaneous disease.

The accidental finding of non-exudative glomerulonephritis in a rat given prolonged sulfapyridine treatment led to the present investigation of this lesion and its probable mechanism.

Method

Nineteen white rats, weighing 200 to 225 gm. each, were employed. Seven animals were subjected to a right partial nephrectomy involving the removal of less than one-half, probably only one-third, of the kidney. Eighteen days later a left nephrectomy was done on these rats and also on a group of five normal rats. Both groups were allowed to recuperate for 1 month. The remaining seven animals were not operated upon.

All animals were maintained on a standard diet, consisting of liberal amounts of dried bread, oats, carrots and Purina dog chow, prior to and during the experiment. The rats were given 50 mg. of sulfapyridine suspended in 0.5 cc. of 15 per cent gum acacia, daily, for the first 6 days, by stomach tube. For the next 3 weeks, except over the week-ends, the dosage was increased to 50 mg. twice daily; and then doubled again to 100 mg. twice daily, for the next 7 weeks.

At the beginning of the experiment and once weekly thereafter, each rat was subjected to cardiac puncture under ether narcosis during which 1.0 cc. of blood was removed for determinations of nonprotein nitrogen and of free and total sulfapyridine.

After 17 to 67 days of administration of the drug, about one-half of the animals died from cardiac tamponade or from anesth
viving rats were killed with ether 11 days after the las
treatment. Paraffin sections of the formaldehyde-fixed

* Received for publication, May 5, 1941.

cut at 2 and 4 μ, and stained with hematoxylin and eosin and with azocarmine. Frozen sections were cut at 10 μ and stained with scarlet red.

A control series of ten rats was subjected to a right heminephrectomy during which somewhat more than one-half of the kidney was removed, followed, after 1 week, by removal of the left kidney. These animals received the same diet as those of the other series. They were given no drug, and were not subjected to any experimental procedure. Five deaths occurred: one on the second, two on the third, one on the sixth, and one on the twenty-eighth postoperative day. The five survivors were killed with ether 40 days after the last operation and sections of the kidneys were prepared as in the other series.

Results

Sulfapyridine-Treated Groups

Grossly, urolithiasis with hydronephrosis was encountered in two rats and dilated ureters in two others.

Histologically, the most advanced lesions consisted of focal proliferation of capsular epithelium with adhesions of the glomerular tuft to the capsule (Figs. 1 and 2). Many of the tufts were swollen and hyalinized and the subcapsular spaces were diminished. With the azocarmine stain, endothelial cells exhibited a homogeneous, dark blue cytoplasm which was continuous with the thickened basement membrane of the tuft capillaries. This thickening was most pronounced in the stalk of the glomerular tuft and frequently exhibited an arborescent pattern. The basement membrane of Bowman's capsule was unaltered except for reduplications of the membrane in the foci of glomerular adhesions (Fig. 2).

Many tufts showed foci of reduced cellularity and also foci of increased cellularity. In the latter, the nuclei appeared bunched and were frequently irregular in shape and hyperchromatic. These nuclei appeared to belong to proliferated epithelial and endothelial cells. The hyaline appearance of the tufts was caused mainly by swelling and hyalinization of these cells. The swollen endothelial cells frequently appeared to obstruct the lumina of capillaries. Such glomeruli were often bloodless.

Cloudy swelling of tubular epithelium associated with cellular disintegration and variable amounts of cellular débris in the lumina of the tubules were frequently found. Although these changes appeared to be of postmortem character in some animals, in others the alterations seemed to be antemortem. No fat could be demonstrated in the tubular epithelium or glomeruli.

Only four animals exhibited the histologic changes suggestive of urolithiasis medicamentosa.[5] These changes consisted of low-grade, subsiding, nonpurulent pyelonephritis with slight to moderate focal interstitial fibrosis and focal dilatation of convoluted tubules. In these regions finely divided fat droplets were demonstrable in the tubular epithelium.

Considerable scarring was encountered in a narrow zone upon the operated surface of heminephrectomized kidneys. Glomerular and tubular changes seen in this region were not considered pertinent.

The most widespread and advanced lesions were found in rats from which one and one-half kidneys had been removed, while animals which had not been operated upon exhibited the least involvement and relatively slight or early lesions. Unilaterally nephrectomized animals occupied an intermediate position relative to the amount and severity of renal involvement. Glomerular lesions were found in five of the seven rats operated upon bilaterally; three of the five operated upon unilaterally; and two of the seven animals not operated upon.

The weekly chemical determinations upon the blood showed occasional, sudden, transient increases of nonprotein nitrogen, up to 143 mg. per 100 cc., and of total sulfapyridine, up to 40.7 mg. per 100 cc., in a number of the animals. It was interesting to note that significant elevations in the blood sulfapyridine preceded elevations in the nonprotein nitrogen, and that after the latter had returned to normal the sulfapyridine level was still considerably elevated.

Non-Treated Control Group

All of the microscopic changes present in the drug-treated animals were also found in the non-treated group (Figs. 3 and 4). The severity and frequency of the lesions were no less than in the drug-treated rats which had subsisted with but one-half of one kidney. Only three of the ten control animals did not show glomerular lesions. Swelling and proliferation of the capsular epithelium were more pronounced; proliferation of the epithelium and endothelium of the tuft more frequent; and irregularity in the distribution of nuclei in the tufts more prominent in the control animals.

Scarring in the operated zone of the kidneys was pronounced. As in the drug-treated animals, focal pyelonephritis was encountered. This was generally more severe in the non-treated rats and was related, no doubt, to the lack of bacterial inhibition. The glomerular changes described in this paper were in regions uninvolved by either scarring or pyelonephritis. There were no vascular changes which could be incriminated in the production of the glomerulitis.

The kidneys of the control animals also showed considerable tubular dilatation associated with cloudy swelling of the lining epithelium.

Discussion

In previous experiments with rats [6] no significant glomerular changes were noted following the experimental production of urolithiasis medicamentosa with sulfapyridine. However, monkeys [7] treated with this drug were claimed to show a thickening of the basement membrane of the glomerular tufts.

The glomerular changes reported in the present experiment have not been seen previously with sulfapyridine or other sulfonamide drugs. The architectural alterations which heretofore have been observed with sulfapyridine, have been merely those incidental to urinary obstruction and to the secondary pyelonephritis. This fact, supported by the occurrence of identical glomerular changes in untreated rats, makes it very unlikely that sulfapyridine was directly responsible for the glomerular lesions. Furthermore, because such lesions were found predominantly in those rats from which one and one-half kidneys had been removed, it appears that glomerular damage was conditioned by a reduction in renal parenchyma. By causing urinary obstruction and thereby a further reduction in the functioning renal parenchyma, sulfapyridine apparently played a contributory rôle.

The fact that considerably more renal parenchyma was removed in the control rats than in the sulfapyridine-treated animals is offered as an explanation of the severity of the lesions in the untreated controls. Reduction in the amount of renal parenchyma was also an important consideration in the production of glomerular lesions by means of high-protein diets.[3] Unilateral nephrectomy was found to be a prerequisite in such experiments. The similarity of the lesions produced by two different methods suggests that the factor common to both methods—reduction in renal parenchyma with probable excessive work-demand placed upon remaining glomeruli—may be the immediate cause of the glomerular changes.

Although surgical reduction of renal parenchyma is not a factor in the glomerulitis produced by nephrotoxic sera, the similarity of the glomerular lesions to those produced by high-protein diets [3] and those described in the present experiments suggests the possibility that perhaps a functional reduction in renal parenchyma is a factor.

The irregular, abrupt, and often pronounced elevations in nonprotein nitrogen and in sulfapyridine content of the blood are adequately explained by urinary obstruction from acetylsulfapyridine uroliths. The return to normal values was caused by spontaneous

solution or passage of the stones. The rise of the sulfapyridine blood level in advance of that of the nonprotein nitrogen level and the persistence of the high sulfapyridine level is explained by reabsorption of this drug from the tubules.

The incidence of uroliths and also of lesions referable to them was surprisingly small in the present experimental series as compared to previous experiments.[6] This discrepancy can be explained only on the basis of spontaneous solution of the uroliths[8] caused by the interruptions in the drug treatments over the various week-ends.

Conclusions

1. The prolonged administration of sulfapyridine in excessive doses was followed by (but not the cause of) glomerular lesions in partially nephrectomized white rats. The greater the amount of kidney tissue removed surgically prior to administration of the drug, the more frequent and the more severe were the lesions which were found.

2. The glomerular lesions consisted of swelling and proliferation of capsular epithelium, thickening of basement membranes of glomerular capillaries, focal hyaline degeneration of the tufts, endothelial and epithelial proliferation in glomerular tufts and focal adhesions of tufts to Bowman's capsule in foci of capsular epithelial proliferation.

3. Identical lesions were produced in rats by the resection of appropriate amounts of renal parenchyma without resorting to additional procedures.

4. Sulfapyridine does not appear to have been directly concerned in the etiology of the glomerulitis in the drug-treated rats. It appears more likely that the production of the lesions was related to the excessive work-demand placed upon the glomeruli remaining after surgical resection of renal tissue and after tubular obstruction by sulfapyridine uroliths.

5. The glomerular lesions observed resemble those caused by nephrotoxic sera or by a high protein diet.

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DESCRIPTION OF PLATE

PLATE 15

FIG. 1. Glomeruli showing proliferation of capsular epithelium and adhesions of tufts to capsule. From a rat treated for 11 weeks with sulfapyridine following removal of one and one-half kidneys. Hematoxylin and eosin stain. × 620.

FIG. 2. Glomerulus showing reduplications of the basement membrane in the region of glomerular adhesion. The basement membrane of the tuft is thickened. From the same animal as Figure 1. Azocarmine stain. × 620.

FIG. 3. Glomerulus showing focal proliferation of capsular epithelium, adhesion of tuft and swelling and hyalinization of the cells in the upper portion of the tuft. From a control rat not treated with sulfapyridine but subjected to removal of somewhat more than one and one-half kidneys. Sacrificed 40 days after the last operation. Hematoxylin and eosin stain. × 620.

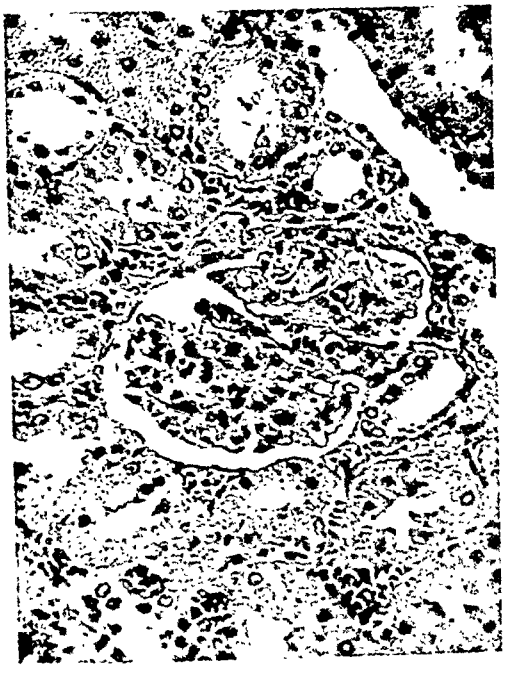
FIG. 4. Glomerulus showing proliferation and swelling of capsular epithelium, adhesions of the tuft, and "bunching" of hyperchromatic, irregularly shaped, shrunken nuclei in the center of the tuft. From the same animal as Figure 3. Hematoxylin and eosin stain. × 620.



1



2



3



4

Gross, Cooper and Morningstar

Glomerulonephritis a

INTERSTITIAL MYOCARDITIS FOLLOWING THE CLINICAL AND EXPERIMENTAL USE OF SULFONAMIDE DRUGS*

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In the routine microscopic examination of autopsy material at the University Hospital (Ann Arbor) during the past 4 years, there were noted occasional examples of interstitial myocarditis, with eosinophilic cellular infiltrations, for which the usual etiologic factors appeared to be lacking. Upon comparing the clinical records of these cases it was found that medication with one or more of the sulfonamide drugs during the terminal illness of each patient was the only factor common to them all. This finding led to two lines of investigation. In one, the autopsy material for the period 1937–1941 was surveyed to determine the incidence of this form of myocarditis in relation to known therapeutic use of the sulfonamide drugs. In the other, daily intraperitoneal introduction of neoprontosil, sulfanilamide, sodium sulfapyridine and sodium sulfathiazole into mice, rats and rabbits, in doses slightly less than those usually employed in clinical medicine, was followed by microscopic examination of the organs of these animals with special attention to the hearts.

LITERATURE

In spite of the voluminous literature relative to the sulfonamide drugs, there is comparatively little histopathologic data available. Observations have been based largely on experimental material and have dealt with the kidneys, liver, spleen and bone marrow. Only a few reports can be found in which the effect of these drugs on the myocardium is considered. Nelson [1] noted myocarditis in a hen which had been given sulfapyridine. The cellular infiltrations were said to be monocytes and polymorphonuclear cells. Eosinophil cells were not mentioned. Dozzi [2] and also Scheinberg and Ingle [3] noted electrocardiographic changes in patients receiving drugs of the sulfonamide group. In each instance, the patient recovered.

Several authors have failed to note changes in the hearts of animals used for toxicologic studies of these drugs. In some of these reports it is not clear to what extent the myocardium was examined histologically. Marshall, Cutting and Emerson [4] reported an absence of histologic changes in dogs, mice and rabbits that had received sulfanilamide. Davis, Harris and Schmeisser [5] gave large doses of sulfanilamide to rats for periods up to 3 months and reported focal necrosis of the liver,

* Received for publication, June 30, 1941.

enlargement of the spleen and sulfanilamide crystals in the exudate in the bronchi. Using rats, Machella and Higgins [6] produced an anemia with sulfanilamide in doses of from 0.25 to 2.0 gm. per Kg. of body weight, given by stomach tube over periods of 10 days to 2 months. The histologic changes were reported as similar to those noted by Davis, Harris and Schmeisser. Molitor and Robinson [7] found no histologic changes with benzylsulfanilamide, but noted splenic enlargement and degenerative changes in the liver and kidneys from sulfanilamide itself. Antopol and Robinson,[8] using sulfapyridine and sodium sulfapyridine in toxic doses, produced urolithiasis in rats, rabbits and monkeys, but not in mice or dogs. Renal histopathology was stressed and no changes were noted in the hearts of the animals examined. Antopol, Lehr, Churg and Sprinz [9] found changes in the livers and kidneys of rats after the intravenous injection of the sodium salts of sulfapyridine, sulfathiazole and sulfamethylthiazole like those previously described by Antopol and Robinson, and others. Stryker [10] found basophilic casts in the renal tubules of animals given sulfapyridine. Toomey, Reichle and Takacs [11] reported similar renal changes in monkeys receiving sulfapyridine in doses comparable to those used in infants. Microscopic lesions were found by Rake, van Dyke and Corwin [12] in the livers and kidneys of mice, rats and monkeys to which sulfathiazole and sulfapyridine were administered with food. Sobin,[13] using large doses of acetylsulfapyridine in rats, produced concretions in renal tubules, as well as dense infiltrations of eosinophils in the submucosal tissues of the renal pelves.

References to the induction of eosinophilic cellular infiltrations and to blood eosinophilia following the use of these drugs have appeared. Hageman,[14] in mice on heavy doses of sulfanilamide, found an increase in the eosinophils of the bone marrow. P'an [15] found eosinophilia of the peripheral blood stream in a dog which had been given large doses of sulfanilamide over a period of several months. However, this dog was found at autopsy to have had tapeworms (*Dipylidium caninum*). Although many organs were studied microscopically, no histologic changes were noted. Kreutzmann and Carr [16] found an increase in the eosinophils of the blood in rabbits on a daily dose of prontosil of 0.25 gm. per Kg. of body weight for 21 days. Interpretation of eosinophilia in rabbits is difficult, but the controls seemed adequate.

Clinical Material

It was ascertained by inspection of the individual case records that 283 of the 1,706 patients upon whom autopsies were performed during the period 1937–1941 had been given one or more of the sulfonamide

drugs during some part of the last few weeks before death. This figure is known to be less than the actual total because a complete record of the medication was not available in every instance. The routine microscopic sections, stained with hemalum and eosin, of the hearts of these 283 patients were reëxamined. In 61 there was no evidence of interstitial myocarditis. These negative findings were not significant in all instances because some of these patients had been given only a few doses of a sulfonamide drug or such medication had so shortly preceded death that it could not have been expected to produce a tissue reaction. Also examination of additional sections from other portions of the myocardium might have reduced the number considered negative. In 40 hearts, interstitial infiltrations were found, but were of so slight a degree that this group was set aside as inconclusive. Infiltrations of this degree were occasionally found without assignable cause before the sulfonamide drugs were introduced. Also excluded from further consideration were 56 hearts from patients in respect to whom there was evidence, either from clinical observation or from the findings at autopsy, of some disease known to result in, or to be associated with, interstitial myocarditis. Thus hearts from cases of bacterial endocarditis, coronary or mural thrombosis, purulent pericarditis, rheumatic heart disease, syphilis, diphtheria, septicopyemia, leukemia, asthma and trichinosis were excluded, although many showed interstitial infiltrations which were rich in eosinophils and were in every respect like those to be described for the group which was retained as significant. After these exclusions there remained a group of 126 cases (44.5 per cent of the original 283) in which a significant interstitial myocarditis was present. For this group, sulfonamide medication was the only factor known to be common to them all. It was therefore believed to be the cause of the myocardial lesion.

When the 56 cases which were excluded from consideration because of the presence of some condition which might produce an interstitial myocarditis are deducted from the original group of 283 cases which had received sulfonamide drugs, the percentage showing an otherwise unexplained myocarditis rises to 55.5.

The patients in this group varied in age from 1 month to 87 years. The acceptable cases were divided about equally as to sex; 70 males and 56 females. The drugs given them included azosulfonamide (prontosil, neoprontosil), sulfanilamide (prontylin), sulfapyridine (dagenan), sulfathiozole and sulfamethylthiazole. The amounts which apparently produced significant infiltrations varied from 5 gm. to more than 200 gm. and were administered over periods of a few hours to several months. No examples of interstitial myocarditis were found in

which the use of the drugs had been discontinued for more than 30 days prior to death. An evaluation of the liability of each particular drug to produce myocardial infiltrations was impossible from the clinical material, for many patients had received several members of the group at different times and for variable periods of time during their terminal illnesses.

As a control series, the hearts from 50 consecutive autopsies performed in 1932 were examined microscopically and all cellular infiltrations noted. This search was conducted without knowledge of either clinical or autopsy records for these cases. The records were subsequently consulted and in this group a factor known to be a causal element in the production of interstitial myocarditis was found to be present in every case in which infiltrations were found. We are fully aware that such a result would not be uniformly obtained because in years past not every case of interstitial myocarditis could be accounted for by one of the conditions used to eliminate cases which might have invalidated the results in the group which received sulfonamide drugs. There have always been cases of "idiopathic" interstitial myocarditis, although this group seems to grow smaller year by year.

The Myocardial Lesion

No macroscopic changes attributable to the use of the sulfonamide drugs were found in the hearts included in this series.

The microscopic features of the lesion in the clinical material included an interstitial myocarditis with the cellular infiltrations chiefly paravascular in distribution but also occurring diffusely in the more marked examples (Figs. 1 and 2). The myocardium was widely involved in both auricles and ventricles. The looser auricular myocardium often exhibited a heavier paravascular infiltration than did the compact ventricular muscle. Some foci were likewise seen in the epicardium, but such cases were included in the positive series only when there was no exudate upon the epicardium and when a characteristic infiltration was found in the myocardium as well.

The cellular infiltrations contained large mononuclear cells of clasmatocytic type and, characteristically, numerous cells with granular eosinophilic cytoplasm. Some of the eosinophils were polymorphonuclear but many appeared to be younger forms of the myeloid series and had a single nucleus and a less granular cytoplasm. Except for the acidophilic cytoplasm, some of them resembled plasma cells.

In occasional hearts the interstitial supporting tissue showed a slight bluish-staining quality with hemalum and eosin. This was considered to be due to mucinous degeneration of connective tissue, but correlation

with either intensity or duration of treatment could not be demonstrated. The significance of this change remained undetermined.

Parenchymatous degeneration in the form of slight Zenker's necrosis was noted but there was no significant simple necrosis and no liquefaction of the muscle. No abscesses were found which could be considered a part of the significant lesion. However, abscess formation was interpreted as evidence of septicemia even though the abscesses were found only in the heart and no positive blood cultures were obtained. Therefore, such cases were automatically excluded from further consideration.

Eosinophilic infiltrations were occasionally noted in *other organs,* particularly in the liver, lungs and kidneys. In some instances the bone marrow, spleen and lymph nodes showed at least a relative increase in eosinophils. The presence of eosinophil cells in these situations may prove to be significant, but no further study of them was made in connection with the immediate problem.

Experimental Production of the Lesion

When the association of interstitial myocarditis, rich in eosinophil cells, with medication with neoprontosil was first noticed in the autopsy material, small groups of mice, rats and rabbits were started upon daily intraperitoneal injections of neoprontosil (the disodium salt of 4-sulfonamido-phenyl-azo-7-acetylamino-1-oxynaphthalene-3:6-disulphonic acid). Dosage was so computed as to be slightly less than the usual human dose of 4 gm. per 24 hours for an adult weighing 70 to 75 Kg. A smaller dose was chosen for experimental introduction in the belief that absorption would be more effective from the peritoneum than from the gastro-intestinal tract. Keeley,[17] however, has found that, although sulfanilamide is absorbed promptly from the peritoneal cavities of dogs, the concentration of the drug in the blood is lower following intraperitoneal injection than other workers have reported as resulting from oral administration. This discrepancy in the expected concentration in the blood has not been explained. It can have no effect upon the significance of the results obtained in the experimental series here reported other than to enhance their value, for the dosage was apparently more conservative than was at first planned and was definitely lower than standard human medication regardless of the mode of introduction.

For the preliminary series, a 5.0 per cent solution of neoprontosil was used for daily intraperitoneal injection in the following amounts: for mice, 0.015 cc.; for rats, 0.15 cc.; for rabbits, 1.5 cc. These animals were sacrificed at intervals and interstitial cellular infiltrations were found in the hearts of many of them.

In view of the positive findings in the preliminary series, larger groups of animals were employed for a second series and sulfanilamide (para-aminobenzene sulfonamide), sodium sulfapyridine (sodium salt of 2-sulfanilamide-6-aminopyridine) and sodium sulfathiazole (sodium salt of 2-sulfanilamide-thiazol) were administered, as well as neoprontosil. The sodium salts of sulfapyridine and sulfathiazole were selected for their greater solubility as compared with the parent products. Neoprontosil, sodium sulfapyridine and sodium sulfathiazole were used in a 5.0 per cent aqueous solution while the less soluble sulfanilamide was in a 0.5 per cent aqueous solution. Daily intraperitoneal injections were made. A small area on the abdominal wall was shaved for an injection site and sterile technic was observed throughout so far as possible. No evidences of either acute or chronic peritonitis were noted at autopsy.

Animals similar to those of the first series were employed. The Swiss mice weighed 20 to 25 gm.; the Wistar stock rats, 200 to 250 gm., and the domestic rabbits of mixed strains, 2,000 to 2,500 gm. All animals used were virginal and males and females were separated to avoid pregnancy as a variable factor. Dosages were computed, as for the first series, to be slightly smaller than the usual human dose. The amount of each drug that was injected at one time is shown in Table I. The

TABLE I

Daily Intraperitoneal Injection Given to Second Experimental Series

	Neoprontosil 5.0% aqueous solution	Sodium sulfapyridine 5.0% aqueous solution	Sodium sulfathiazole 5.0% aqueous solution	Sulfanilamide 0.5% aqueous solution
	cc.	*cc.*	*cc.*	*cc.*
Mice	0.015	0.015	0.015	0.15
Rats	0.15	0.15	0.15	1.5
Rabbits	1.5	1.5	1.5	15.0

mice and rats were killed at intervals of 10, 20 and 30 days after receiving for that period daily intraperitoneal injections of the drug under trial. To additional groups of mice and rats neoprontosil and sulfanilamide were given for 10 days in doses ten times the established sub-normal comparable human dose. Sodium sulfathiazole and sodium sulfapyridine were not available in sufficient quantity for protracted use at this concentration.

As controls, 29 mice, 26 rats and 26 rabbits, all untreated but from the same stock as those treated, and housed and fed in the same manner, were killed and their hearts were examined microscopically. "Spontaneous myocarditis" was found in many of the control rabbits. Miller [15] has reported an incidence of interstitial myocarditis of 60 per cent in control rabbits. Seegal, Seegal and Jost [16] suggested a local

application of the Arthus phenomenon as a possible etiologic factor in the apparently spontaneous interstitial myocarditis of rabbits. These reports, fortified by the findings in our control rabbits, led to the exclusion of the material from rabbits from further consideration. No cellular infiltrations of significance were found in the hearts of either the control mice or rats.

In the second experimental series, an interstitial myocarditis, characterized by numerous eosinophil cells and resembling in all respects that in the clinical material was found in 38 of 60 mice and in 33 of 47 rats. Details as to the duration of administration of the drugs and the distribution of positive findings are shown in Table II. Similar infiltrations were found in 12 of 14 rabbits which were injected at the same time but, as previously stated, these were considered to be of questionable significance.

TABLE II
Second Group

Drug used and duration of experiment		Mice		Rats	
		Total number used	Number with myocarditis	Total number used	Number with myocarditis
Neoprontosil	10 days	4	2	4	3
	20 days	3	4	3	1
	30 days	3	4	3	3
Sulfanilamide	10 days	4	0	4	3
	20 days	3	3	2	2
	30 days	3	3	3	2
Sodium sulfapyridine	10 days	4	4	4	3
	20 days	3	1	2	2
	30 days	3	3	3	3
Sodium sulfathiazole	10 days	4	1	4	3
	20 days	3	3	2	2
	30 days	3	2	3	2
Tenfold dosage					
Neoprontosil	10 days	10	5	10	4
Sulfanilamide	10 days	10	3	0	0
Totals		60	38	47	33

It will be seen that myocardial infiltrations followed the use of each of the drugs administered. In general, the frequency with which myocarditis developed increased with the duration of treatment and with the amount administered. The lesions tended to be more focal in character in the experimental animals than in the human material.

DISCUSSION

As previously stated, no attempt was made to determine from the clinical material whether the various drugs used differed in their effects

upon the myocardium. The use of more than one drug for the same patient and the extreme variability in intensity and in duration of dosage made such an investigation futile. In the experimental series, as they were set up with less than comparable clinical dosage, no significant differences in toxicity were recognized among the four drugs utilized.

No strict correlation between the duration and intensity of medication on the one hand, and the severity of the interstitial myocarditis on the other, could be found in the clinical material. Actually some patients with marked myocardial infiltrations had received relatively small doses of the drugs over short terminal periods in their illnesses. The question of idiosyncrasy to the drugs is thereby raised—a question which seems all the more appropriate in view of the eosinophilic character of the infiltrations. No solution to this question is evident from this work, but a definite avenue of approach is suggested.

That the lesions attributable to sulfonamide drug therapy are not of a character which would be expected to result in irreversible changes is significant and encouraging. Nevertheless, it is impossible to evaluate the effect which such lesions might have on the heart of an individual who was otherwise seriously ill. It is therefore highly desirable that such patients be examined at frequent intervals with reference to their cardiovascular functional efficiency. Electrocardiographic studies might properly parallel sulfonamide drug therapy in many cases.

It is not the intent of this report to discourage the use of the sulfonamide drugs, but to call attention to one of the several by-effects which make it desirable to keep patients, to whom these drugs are being given, under the closest clinical scrutiny and to limit the amount and the duration of administration to the lowest levels consistent with beneficial results.

Summary

An interstitial myocarditis, rich in eosinophil cells, has been found in the hearts of 126 patients whose sole common factor was that one or more of the sulfonamide drugs had been administered shortly before death.

A similar eosinophilic interstitial myocarditis has been produced in mice and rats by the daily intraperitoneal injection of neoprontosil, sulfanilamide, sodium sulfapyridine and sodium sulfathiazole in amounts computed to be less than comparable to the usual human dosage.

Eosinophilic infiltrations were seen also in the lungs, livers and kidneys of both the human cases and of the experimental animals. The

bone marrow, spleen and lymph nodes likewise showed increased eosinophil cells in some instances.

These observations suggest the advisability of greater precaution in the use of the sulfonamide drugs.

NOTE: A preliminary report upon this investigation appeared in *Proc. Soc. Exper. Biol & Med.*, 1941, 48, 1–3. Differences in numerical results in the experimental sections of the two reports are due to combining series in the preliminary report and to slight changes in diagnostic criteria considered to be significant.

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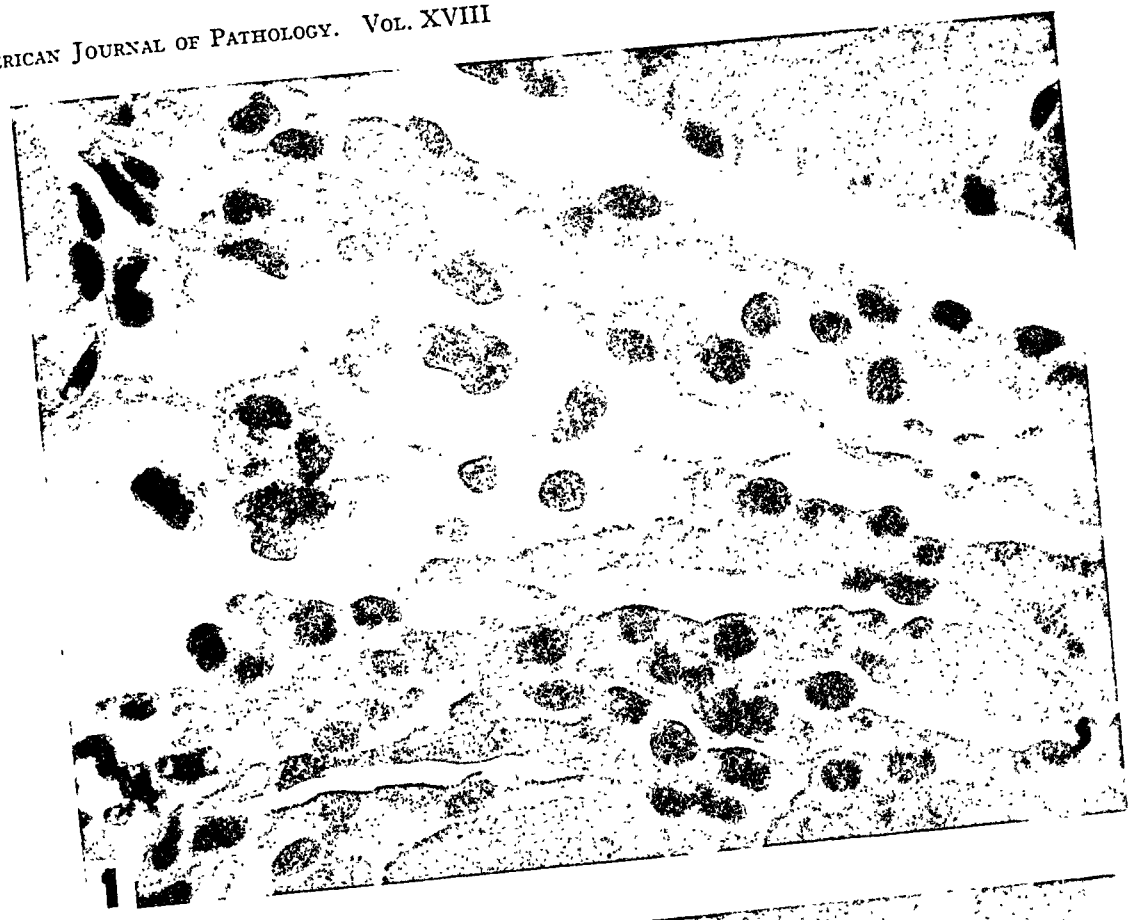
DESCRIPTION OF PLATES

PLATE 16

FIG. 1. Interstitial myocarditis as found in the heart of a woman 48 years old (A-91-AS) who had received 4 gm. of neoprontosil daily for the terminal 4 days of life. This was given in an effort to control a postoperative infection. Hemalum and eosin stain. × 410.

FIG. 2. Interstitial myocarditis as found in the heart of a man of 72 years. For infection associated with prostatic obstruction, he had been given a total of 50 gm. of sulfanilamide during the last 2 weeks of life. Hemalum and eosin stain. × 400.

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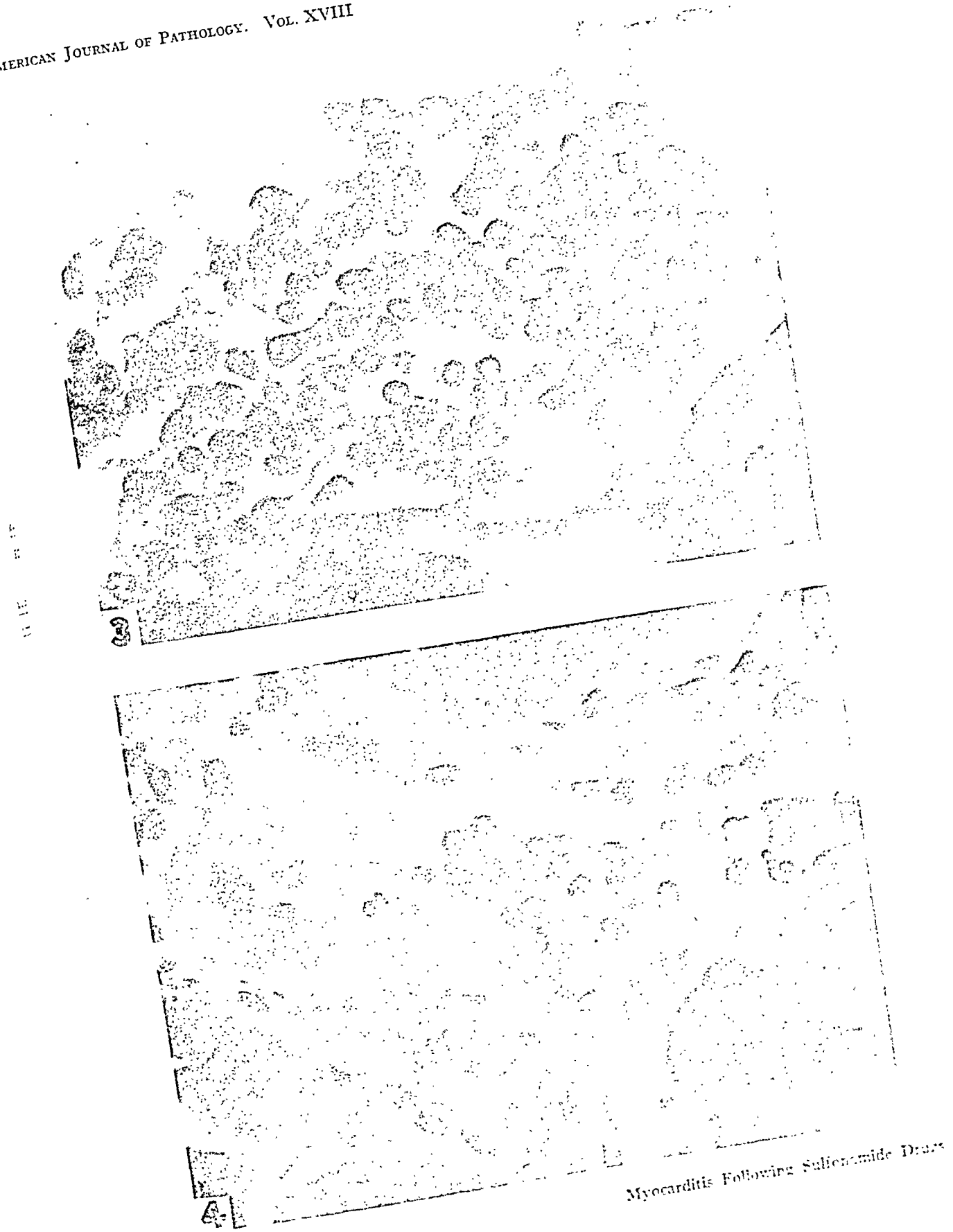
Myocarditis Following Sulfonamide Drugs

French and Weller

PLATE 17

FIG. 3. Subendocardial infiltration photographed from the heart of rat no. 61, which had received daily intraperitoneal injections of sulfanilamide for 10 days. Hemalum and eosin stain. × 390.

FIG. 4. Interstitial myocardial infiltration in the heart of mouse no. 72, which had received daily intraperitoneal injections of neoprontosil for 20 days. Hemalum and eosin stain. × 390.



Myocarditis Following Sulfonamide Drugs

BRONCHIOGENIC CARCINOMA IN ASSOCIATION WITH PULMONARY ASBESTOSIS *

Report of Two Cases

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Following the report of a case of carcinoma of the lung in the presence of pulmonary asbestosis by Lynch and Smith [1] in 1935, several similar papers appeared in rapid succession. In the same year, Gloyne[2] presented two cases of squamous-celled carcinoma of the lung occurring in asbestosis, and in the following year,[3] a case of oat-celled carcinoma. Egbert and Geiger [4] reported one case in 1936. Two years later, Nordmann [5] analyzed the recorded cases after adding two of his own to the literature. His views on the relationship between the two diseases were revealed by the title of his paper, "Der Berufkrebs der Asbestarbeiter" [Occupational Cancer of the Asbestos Worker]. The eighth case was reported by Lynch and Smith [6] together with an additional case of squamous metaplasia of the bronchial epithelium in an asbestotic lung. The two cases here reported bring the total number recorded in the literature to ten.

Reports of Cases

Case 1

W. M., a white male, 52 years old, was admitted to Queens General Hospital complaining of a chronic morning cough which for many years had been productive of small amounts of tenacious sputum. During the 4 weeks prior to admission, the cough became progressively more severe and he was compelled to stop work. The sputum now amounted to approximately 4 oz. (120 cc.) each day and was occasionally blood-streaked. This was accompanied by right chest pain, marked anorexia and a weight loss of about 15 lbs. (6.5 Kg.).

There was no history of past medical, surgical or venereal disease except for a nasal operation at the age of 25.

For the past 25 years the patient had been employed almost continuously by various manufacturers of asbestos products. His duties always entailed the covering of steam and water pipes with asbestos fabric. He stated that many of his co-workers had died of "silicosis."

Physical examination revealed a weakened, emaciated, white male breathing noisily and with difficulty, and coughing frequently. The neck veins were distended. There were signs of consolidation over the upper right portion of the chest anteriorly. The breath sounds were distant over the lower posterior areas on both sides of the chest. The cardiac apex and the trachea appeared shifted to the right. There were no other unusual findings in the heart, abdomen, extremities or nervous system.

Laboratory Data. Temperature, 99.8° F. Pulse, 124 per min. Respirations, 22 per min. Blood pressure, 110/80. Urine, negative except for occasional red

* Received for publication, May 19, 1941.

and white blood cells. Blood count, 4,800,000 red cells per cmm. and 11,000 white cells per cmm., of which 72 per cent were polymorphonuclear cells. Hemoglobin, 12.3 gm. per 100 cc. Venous pressure, 140 mm. of blood. Ether circulation time, 15 sec. Saccharin circulation time, 3 min. (probably unreliable). The sputum was negative for acid-fast bacilli. A Kline test of the blood was negative.

Roentgenograms of the chest taken shortly after admission showed a large area of diminished illumination involving the entire right upper lobe with "patchy infiltration of the lower lobe." The lower cardiac shadow was deviated to the right. The left lung field presented prominent pulmonary markings which, however, could not be regarded as definitely pathological. On the fifth hospital day bronchoscopy revealed a sessile mass protruding from the posterior wall of the trachea about 1.5 cm. above the carina, leaving a narrow crescentic lumen about 2 mm. in width. Biopsy from this region showed bronchial tissue with only an inflammatory reaction. During the next 12 days the patient received small daily doses of high-voltage roentgen-ray therapy to the anterior aspect of the chest, totaling 2,600 r. This was accompanied by a marked improvement in respiration. The intratracheal mass had now been reduced in size sufficiently to allow passage of the bronchoscope into the right main bronchus, where ulceration and constriction were noted. Biopsy from this region showed definite carcinoma.

During the first 4 weeks in the hospital he had a low-grade fever which gradually declined. On the 30th hospital day, his temperature rose to 105.2° F. and he complained of a sore throat. Blood cultures taken at this time and repeated 5 days later showed the presence of hemolytic streptococci. In spite of sulfapyridine and, later, sulfanilamide therapy, his temperature remained elevated (101° to 104° F.). He grew rapidly weaker and expired on the 36th hospital day.

The final clinical diagnoses were bronchiogenic carcinoma in the region of the carina, *Streptococcus hemolyticus* septicemia and terminal pulmonary edema.

Postmortem Findings. The pertinent descriptive data are limited essentially to the thorax.

The right pleural cavity was completely obliterated by dense fibrous adhesions. The left pleural cavity contained approximately 200 cc. of watery yellow fluid with numerous flakes of fibrin. A shaggy yellow coating covered the posterior parietal pleura. Section of the right lung (Fig. 1) revealed an irregular, globular, hard, grayish white mass 5 cm. in diameter. The mass had completely eroded and encircled the right main bronchus. Some portions of the mass were softened and had been partially excavated. Grayish white neoplastic tissue was seen infiltrating beneath intact bronchial epithelium upward to a point 2.5 cm. above the carina, and downward along the right main bronchus for a distance of 5 cm. beyond the lower margin of ulceration. The remainder of the right upper lobe presented several prominent, irregular, grayish white septa extending out to the pleura which measured 3 mm. in thickness in this region, and was composed of firm, opaque, white tissue. The parenchyma of the right upper and middle lobes presented numerous pneumonitic areas. The cut surfaces of the right lower lobe and the entire left lung were grayish red and crepitant.

Within the mediastinum, at the level of the bifurcation of the

trachea, there was an irregular, nodular mass of necrotic neoplastic tissue 8 cm. in diameter which was adherent to and compressed both trachea and esophagus. (This probably corresponded to the mass visualized bronchoscopically.) The pulmonary vessels were intact except for the main branch to the right upper lobe which was so compressed by the intrapulmonary tumor mass that a probe was passed only with difficulty.

Within the pericardial sac there was approximately 300 cc. of fluid similar to that found in the left pleural cavity. A thick, shaggy, yellowish gray coating covered both visceral and parietal pericardium. The myocardium of the right ventricle was 6 mm. thick.

The only extrathoracic metastasis seen grossly was a mass measuring 4 by 2 cm. at the lower pole of the right kidney. Multiple sections of the brain revealed no metastasis. The right adrenal gland showed a cortical adenoma measuring 3 cm. in diameter.

Microscopical study showed that the neoplastic tissue in the lung was composed chiefly of fairly large polyhedral cells with moderately small, round hyperchromatic nuclei. There was no evidence of either intercellular bridges or of "pearl" formation. Many areas showed considerable variation in the size and shape of these cells and in some, large multinucleated cells were present. In a few regions these cells were spindle-shaped and were arranged in cordlike bundles. There was a definite tendency to invade along the alveolar walls, which occasionally gave a superficial resemblance to large glands. Lymphatic permeation was prominent throughout all involved areas. Small islands of neoplastic tissue were present beneath intact bronchial epithelium, corresponding to the picture seen grossly. Many of these islands were in the lumina of distended lymphatics of the lamina propria (Fig. 2).

Small and large masses of the neoplasm, many of which were necrotic, were found within the wall of the bronchus to the right upper lobe, the adjacent lung tissue, the mediastinal lymph nodes and the right kidney. A routine section revealed a minute metastasis lying in the substance of the adrenal cortical adenoma.

Of interest was the presence of squamous metaplasia of the epithelium of the right main bronchus and of several of the bronchial mucous-gland ducts in this region. Distinct intercellular bridges were seen. Structure was slightly atypical, but there was no de
nant change in the metaplastic epithelium.

Many sections of both lungs showed considerable diff
fibrosis, numerous foamy mononuclear cells wit e
and a moderate number of golden yellow a
These varied from rod-shaped "early" for

forms. Many were partially or completely engulfed by mononuclear and multinuclear macrophages. Sections treated with potassium ferrocyanide gave positive results for iron. In several regions the alveoli were filled with polymorphonuclear cells and fibrin. In areas of fibrosis a marked obliterative endarteritis was present.

Chemical examination yielded 240 mg. of silicon dioxide per 100 gm. of dried lung.

The final diagnoses were: Bronchiogenic, non-keratinizing, squamous-celled carcinoma of right upper lobe; metastases to mediastinal lymph nodes, to right kidney and to a cortical adenoma of right adrenal gland; pulmonary asbestosis; right ventricular hypertrophy; squamous metaplasia of bronchus; bronchopneumonia of right upper and middle lobes; left pleural effusion; fibrinous pericarditis (*Streptococcus hemolyticus*) with effusion.

Case 2

The history was obtained from the patient's son after the autopsy was completed. His knowledge of some details was admittedly inaccurate.

E. R., a white male, 58 years old, began to experience dyspnea on exertion approximately 15 years before his death. This progressed slowly but steadily so that during his last few years of life he was almost completely exhausted after climbing only one flight of stairs. He had frequent bouts of coughing which were especially severe in the morning but occurred throughout the day and night. This was productive of moderately large amounts of sticky brown sputum which was never blood-streaked. During the spells of coughing, "wheezing" noises in his chest could be heard many feet away. After several years, he was examined by a physician who made a roentgenogram of his chest and told him he had "asbestosis of the lungs." There was no history of cyanosis, orthopnea, paroxysmal dyspnea, angina pectoris or ankle edema.

Several months before his death the patient began to lose weight rapidly. He complained of continual fatigue and his cough became more frequent and more severe. There was no change in the character of his sputum. A physician told the family that the patient had developed "complications following asbestosis." He sank slowly, became comatose and finally expired.

The patient had been employed by a manufacturer of asbestos products since the age of 23 years until a few months before his death. During the first 25 years of this period he was employed as a pipe-coverer, the same occupation as in case 1. Although there was no known association between the two men, the conditions under which they worked were essentially the same. The patient's work called for covering steam and water pipes with asbestos. He was seldom in the same place for more than a few days or weeks, but the work usually was done in unventilated cellars. Large amounts of powdered asbestos cement were used in addition to the asbestos fabric. The patient's son worked with his father for a year but quit because the air was "too dusty" and because several of the employees were "getting sick." During the 10 years before his death, the patient was employed as a salesman and was not exposed to asbestos dust.

Postmortem Findings. Both pleural cavities were almost completely obliterated by fibrous adhesions between the pleural surfaces. The cut surface of the right lung presented a diffuse infiltration of firm white neoplastic tissue replacing most of the lower lobe. There was prominent encirclement of the bronchi and invasion of the septa by the new growth (Fig. 4). Posteriorly, the adjacent tissue of the right upper lobe showed similar parenchymal, peribronchial and septal infiltration by neoplasm. At the apex of the right upper lobe there was an area of fibrous induration and distortion. No actual specific primary bronchial site could be established, but the major location of the tumor-tissue suggested origin in the right lower lobe.

The remaining lobes of both lungs presented numerous, small, firm, irregular greenish black nodules.

Numerous metastatic foci were present beneath the pleura of the right lung, in the mediastinal and peripancreatic lymph nodes, cerebral cortex, liver, kidneys, thoracolumbar vertebrae and several ribs.

No other noteworthy lesions were present in the remaining viscera.

Microscopical Study. The neoplasm was composed of moderately large, round to oval cells with round, dense, hyperchromatic nuclei. There was considerable variation in the size and shape of the cells with varying amounts of cytoplasm. Many cells contained several nuclei. There was a compact grouping of the neoplastic cells between fine fibrous trabeculae producing a somewhat alveolar arrangement. No true glandular or squamous features were noted.

In the sections of bronchi studied, no ulceration of the mucosa was present. In many regions, however, there was infiltration of tumor-tissue into the lamina propria with invasion and destruction of the remainder of the bronchial wall (Fig. 5). Many of the large neoplastic masses were necrotic. Lymphatic permeation was prominent in all sections. The metastases presented similar histological and cytological characteristics throughout, but the multinuclear tendency was more marked in the liver. The carcinoma cells in the cerebral cortex were less compactly arranged and showed extensive necrosis and hemorrhage.

Sections of lung showed a widespread interstitial fibrosis somewhat unevenly distributed. Only occasional asbestosis bodies were found (Fig. 6). They were smaller but similar to those seen in case 1. Only a few were seen within large macrophages. Sections treated with potassium ferrocyanide were positive for iron.

Additional findings in the lungs included marked hypertrophy of the muscular coat of the small and large bronchi, obliterative pulmonary endarteritis with recanalization, pulmonary edema and organizing fibrinous exudate on the pleural surfaces.

TABLE I

Summary of Reported Cases of Pulmonary Asbestosis and Carcinoma

Authors	Year	Sex and age	Occupation	Duration of exposure	Freedom from exposure before death	Nature of tumor	Primary site	Metastases
1. Lynch and Smith	1935	M 57	Weaver	21 yrs.	4 mos.	Squamous-celled	Right lower lobe	Many nodules in right lower lobe
*2. Gloyne	1935	F 35	Spinner	8 yrs.	9 yrs.	Squamous-celled	Right upper lobe	Pleura
*3. Gloyne	1935	F 71	Mattress and opening depts.	19 mos.	15 yrs.	Squamous-celled	Right lower lobe	None
4. Egbert and Geiger	1936	M 41	Weaver	17 yrs.	2 yrs.	Glandular	Left lower lobe	Widespread
*5. Gloyne	1936	M 59	Packer, stores department	10½ yrs.	? mos.	Oat-celled	Left lower lobe	Left upper lobe and pleura
*6. Nordmann	1938	F 35	Carder, spinner, weaver	7 yrs.	9 yrs.	Squamous-celled	Left lower lobe	Liver, kidneys
*7. Nordmann	1938	M 55	Pre-spinning assembly room	7 yrs.	12 yrs.	Squamous-celled	Left lower lobe	Widespread
8. Lynch and Smith	1939	M 50	Weaver	13 yrs.	3 yrs.	Squamous-celled with glandular features	Right lower lobe	Pleura, mediastinal nodes
9. Holleb and Angrist	1941	M 52	Pipe insulator	25 yrs.	9 wks.	Non-keratinizing squamous-celled	Right upper lobe	Mediastinal nodes, kidney, adrenal
10. Holleb and Angrist	1941	M 58	Pipe insulator	25 yrs.	10 yrs.	Oat-celled	Right lower lobe	Widespread, including brain

*Included in previous tabulation by Nordmann.

Chemical examination yielded 4.5 mg. of silicon dioxide per 100 gm. of dried lung.

The final diagnoses were: Bronchiogenic, large oat-celled carcinoma of the right lower lobe with extension to right upper lobe and metastases to mediastinal and peripancreatic lymph nodes, liver, kidneys, cerebral cortex, ribs and thoracolumbar vertebrae; pulmonary asbestosis; chronic bronchitis; tuberculous apical scar of right upper lobe.

Comment

No reference could be found in the literature providing strict criteria for the diagnosis of pulmonary asbestosis. Silica has been shown [7] to constitute a significant, though somewhat variable, portion of the asbestos fiber (39 to 51.5 per cent). Determinations of silica were therefore carried out with the idea that the demonstration of definitely increased values would furnish final confirmation of the diagnosis of asbestosis. This was considered justified since there was no reason, on the basis of clinical record or autopsy findings, to suspect appreciable exposure to any of the silicates other than asbestos.

The dried lungs of case 1 were found to contain 240 mg. per cent of silica, while those of case 2 yielded only 4.5 mg. per cent. McNally [8] and Kettle and Archer [9] stated that lungs may contain as much as 200 mg. per cent without showing histological evidence of silicosis. If this value were used as a standard, the silica content in case 1 would be only slightly elevated while case 2 definitely would fall within known nonpathological limits. The diagnosis of pulmonary asbestosis in both cases must, therefore, rest on the usual criteria: (1) A history of prolonged exposure to the dust—25 years in both cases; (2) the presence of chronic respiratory symptoms, productive cough in one and cough and severe exertional dyspnea in the other without cardiac disease; (3) histological findings of asbestosis; namely, diffuse, interstitial, pulmonary fibrosis, asbestosis bodies, macrophages and giant cells.

The apparent discrepancy between the presence of pulmonary asbestosis and the absence of elevated silica values can be resolved. First, it is known that inhaled asbestos fibers undergo dissolution and phagocytosis, and may eventually disappear entirely, leaving behind irreparable damage.[10] An additional, though probably minor factor, is the elimination of asbestos by expectoration. This occurs not only as fibers but in the form of asbestosis bodies, showing that a tissue reaction has already occurred in the lung.[11] Second, both of these individuals were employed for unusually long periods in occupations in which the exposure to asbestos dust was relatively low. It is therefore conceivable that extensive damage could result as a summation effect,

without appreciable accumulation of asbestos in the lung at any one time. The combination of both factors may account for the particularly low silica content in case 2 in which there was, in addition, freedom from exposure for 10 years before death.

Nordmann [5] presented in tabular form the relevant data on his own two cases of pulmonary asbestosis and carcinoma, and of the four cases previously recorded. After analyzing the results, he came to the conclusion that pulmonary cancer is an occupational disease among asbestos workers. Gloyne,[12] in a recent text, stated that no definite conclusions can be drawn on the relation between these two diseases until more material is available. Table I presents a tabulation similar to Nordmann's, but brought up to date by the addition of four cases, including the two here reported. It should be made clear that the data are presented merely as a summary and not necessarily as supportive evidence for Nordmann's conclusions.

It may be of interest to review briefly, for comparison, the incidence of carcinoma in the material examined in this department. The figures are derived from the autopsy material of a general city hospital for acute illnesses, with 696 beds of which 498 are devoted to adults. In a total of 2,451 routine autopsies performed in a 5-year period, there were 401 instances of carcinoma of all types. This is an incidence of 16.36 per cent. Of these, 59 were bronchiogenic, comprising 14.71 per cent of all carcinomas and 2.41 per cent of the routine autopsies. It was striking, although naturally of no statistical significance, to find bronchiogenic carcinoma in the only two cases of pulmonary asbestosis seen here.

Conclusions

1. Two cases of bronchiogenic carcinoma in association with pulmonary asbestosis are presented.

2. An attempt to demonstrate unusual amounts of silica in the lungs of both cases was unsuccessful.

3. An explanation for the absence of elevated silica values in the presence of asbestosis is offered.

4. The relevant data of all reported cases of pulmonary asbestosis and carcinoma are presented in tabular form.

Note: The authors wish to express their grateful appreciation to Goodwin A. Distler for the clinical data and to Richard Grimes and Howard W. Neail, of the Office of the Chief Medical Examiner, for the opportunity to study the autopsy material.

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DESCRIPTION OF PLATES

PLATE 18

FIG. 1. Case 1. Hemisected right lung showing ulceration of main bronchus, partially excavated tumor mass in upper lobe, submucosal extension along bronchus, and invasion along interlobular septa.

FIG. 2. Case 1. Right main bronchus showing infiltration of tumor beneath mucosa. × 117.

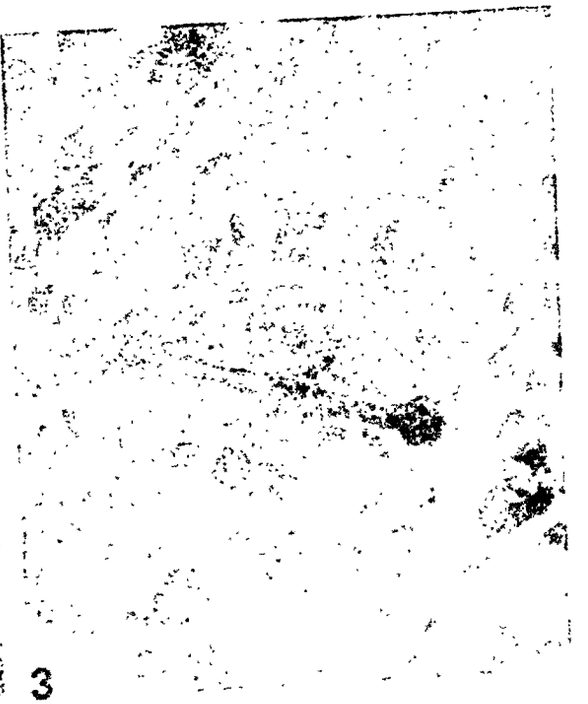
FIG. 3. Case 1. Large asbestosis body with partial encirclement by giant cell. × 525.



1



2



3

Holleb and Angrist

Bronchiogenic Carcinoma and Pulmonary Asbestosis

Plate 19

Fig. 4. Case 2. Right lung showing diffuse carcinoma involving lower lobe and adjacent portion of upper lobe, with prominent encirclement of bronchi by neoplastic tissue.

Fig. 5. Case 2. Bronchus of right lower lobe showing intact basement membrane with attached epithelial layer and underlying diffuse carcinomatous invasion. × 122.

Fig. 6. Case 2. Lung showing several asbestosis bodies. × 547.

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6

Bronchiogenic Carcinoma and Pulmonary Adenomatosis

SECONDARY CARCINOMA IN CIRRHOSIS OF THE LIVER *

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This communication reports six cases of secondary carcinoma occurring in livers affected by diffuse cirrhosis. In each instance, the extent of the cirrhosis excluded the possibility that it was associated with the malignant disease and supported the interpretation that it preceded the presence of secondary nodules.

A careful search of medical indexes revealed only five authentic cases of malignant disease metastasizing to cirrhotic livers. The first case was observed by Hanot and Gilbert.[1] Their first impression was that the nodules in the liver were metastatic, but because such a lesion was so rare in their experience they reported the case as one of primary carcinoma arising in Laënnec's cirrhosis. Poulain [2] restudied the case and came to the conclusion that the hepatic tumor was metastatic. The patient was a woman, 68 years old, who had gastro-intestinal complaints without vomiting or hematemesis for 1 year. A midline mass was present in the upper abdomen. Necropsy revealed carcinoma of the stomach, lymphatic metastases and Laënnec's cirrhosis with malignant nodules. The gastric and hepatic tumors were identical, columnar-celled carcinomas. Poulain was the first observer to differentiate clearly between primary and secondary malignancy in cirrhosis of the liver. White [3] reported the second case. The patient was a man, 63 years old, with sarcoma of bones, on whom he performed an autopsy in 1885. The case of Achard and Laubry [4] was that of a man, 65 years old, a chronic alcoholic, who gave a history of ascites and digestive disturbances and who later developed alternating diarrhea and constipation. Carcinoma of the ascending colon, metastases to lymph nodes, mesentery and omentum, and Laënnec's cirrhosis with tumor nodules were disclosed by autopsy. The original growth and the hepatic nedules were diagnosed as cylindrical-celled carcinoma. The hepatic metastases were within the fibrous tissue in which bile ducts were discernible and distinct from the malignant growth. Two cases were reported by Colwell.[5] The first was that of a man, 69 years old, with malignant disease of the orbit who had a solitary metastasis. In the second case, that of a woman, 58 years old, who had mammary carcinoma, two metastases were present. The cirrhosis in both instances was of the Laënnec type.

* Received for publication, March 31, 1941.

Reports of Cases

Case 1

The clinical data are not available. Necropsy (no. 434) revealed intense jaundice, Laënnec's cirrhosis, carcinoma of the papilla of Vater, metastases to the liver and acute cholangitis. The metastases were largely in the connective tissue.

Case 2

The patient was a white woman, 55 years old, with carcinoma of the stomach who died from hematemesis. Necropsy (no. 3635) revealed carcinoma of the stomach with metastases to liver, regional lymphatics, adrenals, duodenum and transverse colon. The liver weighed 1850 gm. and showed biliary cirrhosis and metastases replacing hepatic parenchyma.

Case 3

The patient was a white woman, 55 years old, who gave a history of obstructive jaundice with operation for gallbladder disease 2½ years before coming under observation at City Hospital. She died of peritonitis following an exploratory laparotomy at which carcinoma of the head of the pancreas was discovered. At necropsy (no. 4262) the liver showed diffuse biliary cirrhosis and extensive small metastases in the fibrous tissue extending into the hepatic nodules. Proliferating bile ducts were visible between the malignant masses.

Case 4

The patient was a white woman, 60 years old, having cirrhosis of the liver and severe anemia. At necropsy (no. 5035) general peritonitis due to a ruptured duodenal ulcer was found. The liver was hobnailed and the inferior surface was attached to the head of the pancreas and the surrounding retroperitoneal tissues by dense, hard, white tissue. The head of the pancreas contained a carcinomatous mass which infiltrated the surrounding tissues and extended directly into the liver. In addition there were numerous very small metastases scattered throughout the liver and extensive involvement of the aortic lymph nodes.

Case 5

The patient was a white man, 82 years old, who was under observation on two occasions, presenting the symptomatology of hepatic cirrhosis with recurring ascites. Necropsy (no. 5537) revealed carcinoma of the stomach and metastases to the pancreas, adrenals and liver. The liver weighed 800 gm. and showed a typical Laënnec's cirrhosis. The metastases were present both in the fibrous portions and replacing hepatic parenchyma.

Case 6

The patient was a white man, 64 years of age, a chronic alcoholic, with carcinoma of the esophagus. Necropsy (no. 5696) revealed squamous-celled carcinoma of the esophagus with extensive thoracic and abdominal lymphatic metastases, and metastases to the omentum and liver. The liver weighed 2150 gm. and showed a typical Laënnec's cirrhosis and squamous-celled metastases replacing hepatic nodules.

Discussion

The rarity of the occurrence of metastatic malignancy in hepatic cirrhosis has been noted by several observers (Hanot and Gilbert,[1] White,[2] Rolleston and McNee [6]). Colwell [5] found only two cases in a series of 2634 autopsies upon patients with malignant disease performed at the Middlesex Hospital between 1854 and 1900. At City Hospital, Welfare Island, 6036 autopsies were performed between May

11, 1911, and December 31, 1940. Of these, 782 were upon patients with malignancy among whom there were 612 with carcinoma, exclusive of 25 examples of primary carcinoma of the liver. The six cases reported in this communication were the sole instances of metastases to cirrhotic livers.

The infrequent occurrence of hepatic cirrhosis in carcinoma of other organs offers one explanation for the rarity of metastases of the liver. Rolleston and McNee [6] noted that with intra-abdominal cancer, cirrhosis was unusual. In Colwell's [5] series, there were 27 instances divided as follows:

Males	*Females*
Mouth and face, 6	Breast, 6
Stomach, 3	Uterus, 6
Orbit, 1	Colon, 1
Testis, 1	
Penis, 1	
Kidney, 1	
Inguinal gland, 1	

In our series there were 22 instances of extrahepatic carcinoma with cirrhosis. The primary sites were as follows:

Kidney, 1	Lung, 1
Duodenum or ampulla, 4	Ovary, 1
Skin of neck, 1	Descending colon, 1
Tongue, 2	Splenic flexure, 1
Stomach, 5	Rectum, 1
Pancreas, 3	Esophagus, 1

Another explanation which may be advanced is that the cirrhotic liver is an unfavorable soil for growth of transplanted tumor cells. The predilection for secondary growths to involve the liver is well recognized. Willis [7] found metastases in 36 per cent of 323 patients with carcinoma upon whom he performed very careful autopsies. He also quoted an incidence of 33 per cent in a summary of data from Colwell,[5] Rolleston and McNee,[6] Kaufmann [8] and Kettle.[9] In our series of 612 cases of carcinoma, hepatic involvement occurred in one-third. Among 27 cirrhotic livers, however, Colwell found only 2 instances, or 7.4 per cent. We found metastases in 6 of 22 cirrhotic livers, a percentage of 27.2. This figure is somewhat lower than that for the entire series of carcinoma but within the range found by other observers (Kettle). It was of interest to note that of the 5 cases of gastric cancer with cirrhosis, 2, or 40 per cent, had metastases. This incidence is almost the same as that found by Willis (44 per cent) in the gastric malignancies in his series. However, since the material is so scanty, figures based on it must be taken with great reserve. If the cirrhotic liver *per se* were an unfavorable soil, one might expect to find cancerous emboli undergoing degeneration and not producing metastases. In

the 18 non-malignant cirrhotic livers such an observation was not made.

The method of involvement of the cirrhotic liver by carcinoma appears to be primarily hematogenous and does not differ from that of the normal liver. However, lymphatic metastasis or permeation is strongly suggested in cases 1 and 4. As Willis [7] has noted, once the liver is affected by secondary growth, the route by which the implant reached the organ is difficult to identify. There was a single example of invasion by contiguity.

The clinical course offers little aid in the diagnosis of hepatic involvement. In three cases (cases 2, 3 and 6) the malignant disease determined the syndrome. In two others (cases 4 and 5) the cirrhosis produced the predominant symptoms. In the cases of Poulain [2] and of Achard and Laubry,[4] the early symptoms were those of cirrhosis, the late symptoms being due to the malignancy.

Summary and Conclusions

Six cases of metastatic carcinoma affecting cirrhotic livers are reported. Only five other cases of metastatic malignancy in cirrhosis were found in the literature. The occurrence of extrahepatic malignancy and hepatic cirrhosis in the same case is uncommon. The liver may be involved by the hematogenous or lymphogenous routes or by direct contiguity. The rarity of metastatic malignancy in a cirrhotic liver may be due either to the unusual association of extrahepatic cancer and cirrhosis, or because the cirrhotic liver is an unfavorable soil for growth of transplanted tumor cells. The clinical course offers little aid in the diagnosis of hepatic metastases.

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NEUROBLASTOMA, GANGLIONEUROMA AND FIBRONEUROMA IN A STILLBORN FETUS *

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Recently, at the Chicago Lying-in Hospital, a stillborn fetus was delivered which exhibited a remarkable neoplastic involvement of the sympathetic nervous system and a generalized hyperplasia of the spinal nerves. The wide dissemination of tumor tissue, the great variation of cellular pattern and the location in certain regions where tumors of sympathetic origin have not been previously described (urinary bladder) make the case of unusual interest. The neoplastic growths include three varieties of tumors which are usually found arising individually in the spinal or sympathetic nervous system; they are neuroblastoma, ganglioneuroma, fibroneuroma.

REPORT OF CASE

Maternal History

Mrs. K., a primipara, 28 years old, had a normal prenatal course until the 27th week at which time she developed a progressive polyhydramnios. She was admitted to the hospital at the end of the 28th week and shortly thereafter went naturally into labor. Presentation was by breech and delivery progressed normally until difficulty was encountered in attempting to deliver the head. A diagnosis of hydrocephalus was made, a craniotomy was performed and the head was easily delivered following evacuation of the cranial contents. The subsequent maternal course was uneventful.

Gross Postmortem Examination

External Surface. The body (no. FA-38-568) was that of a white male infant weighing 1385 gm. and having a crown-heel length of 37.5 cm., a crown-rump length of 26.5 cm. The skin was normal except for postmortem discoloration. The second finger on each hand showed a contracture of the first phalangeal joint due to shortening of the flexor tendons. Both feet were clubbed and were in an equinovarus position.

Head. The head was large in proportion to the rest of the body but the fontanelles were of normal width and the bones of the calvarium seemed of normal thickness. A craniotomy had been performed and practically all of the brain tissue had been washed away. The base of the skull appeared normal except for slight flattening of the fossae. The buccal, pharyngeal and nasal cavities were normal.

* Received for publication, March 24, 1941.

Neck. The trachea was normal. The thyroid lay in its usual position and was entirely normal in appearance. There were several nodular masses of neoplastic tissue lateral and partially anterior to the trachea. The largest involved the vagus nerve on the left side of the neck. Immediately below its exit from the skull the vagus nerve swelled into a bulbous tumor mass slightly more than 1 cm. in diameter. Inferiorly this became fusiform and merged with the hyperplastic nerve trunk. On the left, behind the vagus nerve and the great vessels of the neck, was another similar round mass from which numerous nerve fibers extended. Two tumor nodules were also present on the right and were similar to those on the left except that they were somewhat smaller.

Thorax. All of the organs in the thoracic cavity were displaced upward as a result of subdiaphragmatic pressure, and were compressed against the anterior thoracic wall by the great enlargement of the spinal sympathetic ganglia in the posterior part of the chest. The ganglia extending along both sides of the vertebral column had undergone a neoplastic change and were part of an almost continuous mass of tumor tissue which involved all of the ganglia from the upper cervical to the lower sacral region. This nodular cordlike mass averaged over 1 cm. in diameter. The heart, lungs and thymus were normal except for changes in shape and location caused by compression from the tumors.

Abdomen. The abdominal viscera were displaced upward and anteriorly as a result of pressure exerted by a large tumor of the urinary bladder and the tumor masses in the posterior part of the abdomen. Extending along both sides of the spinal column was the neoplastic involvement of the sympathetic ganglia previously described. In addition there were two large tumors lying behind, but confluent with, the adrenal glands, the one on the left measuring 6 by 6 by 4.5 cm., the one on the right, 5 by 4.5 by 3 cm. They were connected across the midline in front of the aorta by similar tissue. There were also numerous small nodules throughout the abdominal cavity. The tumors in the region of the adrenals were light gray with numerous irregular scattered areas of hemorrhage and calcification. Those in the sympathetic chain were lighter in color, more uniform in appearance and contained much less calcium. A tumor of a third type was found in the bladder wall. It was of firmer consistency and grossly seemed to be composed of nerve fibers which were divided into bundles by intervening connective-tissue fasciculi. This tumor was confluent with the posterior wall of the bladder but did not invade the mucosa. It extended to the umbilicus above, posteriorly encircled the sigmoid colon

and rectum and finally terminated in the hypogastric plexus. It measured 4 by 6 by 6 cm.

The liver occupied the upper abdomen and was normal in size and shape. Immediately beneath the capsule and scattered throughout the substance were innumerable, small, pale yellow areas which appeared to be minute foci of tumorous tissue. The gallbladder and bile ducts were normal. The spleen appeared normal and contained no visible tumor tissue. The gastro-intestinal tract was normal throughout its entire length. In the mesentery of both the large and the small bowel there were many extremely hypertrophied nerves. The pancreas was normal although numerous extremely large nerves traversed the upper border. Both kidneys were flattened in the anteroposterior diameter from pressure exerted by the tumors involving the adrenal glands. A moderate degree of bilateral hydronephrosis was present although the ureters were of normal caliber. The testicles were undescended but appeared normal.

Microscopic Examination

Lung. The alveoli exhibited the pattern which is normal for a fetus which has never breathed. The only pulmonary abnormality consisted in the presence of a small group of neoplastic cells in an interlobular space at the level through which one section was taken. No point of attachment was visible.

Liver. The hepatic cells were well preserved and appeared normal. Diffuse extensive erythropoiesis was present. In all sections examined there were groups of tumor cells scattered throughout the parenchyma which appeared to be proliferating locally. There was no uniformity in distribution in relation to the architecture of the lobules. The cells had round, dark nuclei, pale cytoplasm and the cell boundaries were poorly demarcated. They were similar in character to the sympathicoblasts found in the adrenal tumors.

Pancreas. The islands of Langerhans, acini, and general pattern of the pancreas were normal. The nerves in its substance were increased in size and were distinctly abnormal in form, the central portions being made up of relatively acellular edematous tissue, with only the periphery showing the usual compactness of structure.

Spleen, kidneys, cardiac muscle, thymus, thyroid, testicles and *hypophysis* were normal.

Tumor of the Vagus Nerve. This tissue was an irregular mixture of cells in all stages of differentiation from sympathogonia to mature ganglion cells. Interspersed were large irregular areas of fine fibers arranged in interlacing bundles. They were pink with a hematocytic

eosin stain, blue with azo-carmine and black with silver stains. Very few nuclei were present in these areas. The fibers were similar histologically and in staining reaction to those derived from the sheath of Schwann. In a thin band around the periphery of the mass the cells and fibers were almost entirely mature and the appearance suggested that they may have been the original cells of the ganglia, with only the central areas comprising the neoplasm. The nodule was retort-shaped, the arm of the retort becoming continuous with the inferior portion of the nerve. This part was composed largely of fine fibers similar to those described above.

The other *tumors of the sympathetic ganglia* varied in structure. Grossly they ranged from a pale creamy white to deep gray, and histologically the light-colored ones had in general the same appearance as the nodule on the vagus nerve, while the darker ones bore more resemblance to the masses in the region of the adrenals. The latter contained large groups of sympathogonia with interlacing areas of fine fibrils. Although ganglion cells in all stages of differentiation were present, they were proportionately fewer than in the lighter colored tumors.

Adrenal Tumors. The masses in the region of the adrenals, those forming the connection across the midline, parts of the enlarged sympathetic chain and scattered nodules in the abdomen had a similar structure. They were composed of cells having a fairly uniform pattern, indistinguishable cell boundaries, and containing large, round nuclei showing coarsely distributed chromatin. They were divided into islands by bands of cells containing a large amount of fibrillar material, cells which resembled those of the sheath of Schwann. In some areas there was a tendency for the sympathicoblasts to attempt differentiation into ganglion cells, and in some places calcification and necrosis had occurred. The adrenal glands were still distinct structures although masses of tumor cells were present in the cortex and in the central portions. It is impossible to be certain whether the tumors arose within the glands, whether the cells within them were secondary growths, or whether all developed at the same time.

Bladder Tumor. This bore a close resemblance to a typical neurofibroma. It was composed almost entirely of fibers which were divided into large fasciculi by intervening masses of connective tissue. A few pale, ovoid nuclei were scattered among the fibers and the general appearance was similar to those tumors described as neurofibromas. In a few areas there were very small isolated groups of ganglion cells in various stages of differentiation.

Peripheral Nerves. All of the nerves in the body, both sympathetic

and spinal, were increased in size. There was no increase in neurons and possibly even a decrease, but the hypertrophy was due to a generalized increase in Schwann cells and to edematous, relatively acellular, areas.

Discussion

Tumors of the sympathetic nervous system have been recognized since the middle of the last century. The first group to be studied were the ganglioneuromas, tumors composed of mature ganglion cells and nerve fibers. Since the cells are similar in appearance to those normally found in sympathetic ganglia, a nervous origin for these tumors was quickly accepted. The tumors may be located in any part of the central, peripheral or sympathetic nervous system, or the adrenal gland, but they are found in the region of the paravertebral sympathetic ganglia more commonly than in other locations.

Neuroblastomas, adrenal neoplasms composed of small round cells with deeply staining nuclei, had been commonly regarded as sarcomas until Wright [1] (1910) summarized the literature concerning these tumors and stated the reasons for believing that they originate from cells of the embryonic sympathetic nervous system. Since then, many similar tumors have been observed and approximately 300 have been formally reported as neuroblastomas.

Pheochromocytomas most frequently originate in the adrenal gland but are considerably less common than neuroblastomas or ganglioneuromas. They also may be found in sympathetic ganglia or any place where chromaffin tissue normally occurs. The tumors are composed of mature chromaffin cells and the hormone normally produced by the adrenal medulla has been extracted from them.

Neurofibromas, although usually not classified with tumors of the sympathetic system, may arise from sympathetic nerves as well as from those of the cerebrospinal system and consist of a neoplastic proliferation of Schwann cells. Since Schwann cells, like ganglion cells and chromaffin cells, have differentiated from migratory cells from the neural crest, there is a close interrelationship of all three.

The origin and varying degrees of differentiation found in tumors of sympathetic nervous systems are easily explained on the basis of embryologic development. During the early growth of the embryo, cells migrate from the neural crest to various parts of the organism where they become permanently located and develop into sympathetic ganglia, Schwann cells or chromaffin tissue. These primitive cells are rarely seen in ganglia after the first few weeks of intra-uterine development but may be found in the adrenal cortex throughout fetal life

and may occasionally persist after birth. The fact that cells in this location undergo morphologic and physiologic differentiation later than the other cells of the nervous system may be a contributing factor to the frequent localization of tumors composed of primitive nerve cells in the adrenal glands.

At the time of migration from the neural crest, sympathetic nerve cells are histologically similar to those of the central nervous system and differ only in potentiality for future development. They are small cells whose hyperchromatic nuclei are surrounded by a thin layer of cytoplasm, or are enmeshed in a cytoplasmic mass whose individual cell boundaries cannot be distinguished. Tumors composed of such cells are known as neuroblastomas or more accurately as sympathogoniomas, sympathogonia being the term for entirely undifferentiated sympathetic cells.

Sympathogonia, in the normal process of growth, develop larger, slightly vesicular nuclei, the chromatin becomes clumped, nucleoli appear, and the cytoplasm increases in amount. The cells in this stage are called sympathicoblasts and the tumors composed of such cells, sympathicoblastomas. The term neuroblastoma, as usually used, includes both groups of tumors. Although few fibers are seen under normal conditions at this stage of differentiation, large masses of fibers may be seen in both sympathogoniomas and sympathicoblastomas.

In further development the sympathicoblasts follow one of several possible courses; part become the chromaffin cells, part become the ganglion cells and part develop into Schwann cells. In neoplasms arising from sympathicoblasts, similar lines of differentiation may be followed and the cells may mature into ganglion, Schwann or chromaffin cells. Tumors containing these cells are known as ganglioneuromas, neurofibromas and pheochromocytomas, respectively. All exhibit an extremely variable pattern as a result of the differences in degree of maturation attained in individual tumors, and also as a result of the difference in the relative proportions of cells and fibers which are present.

One would expect that a mixture of ganglion and pheochrome cells would occasionally be found in the same tumor, but this appears to occur very rarely. One of the few instances in which neuroblasts, ganglion cells and chromaffin tissue were all present was reported by Wahl[2] (1914) and was located in the mesentery near the left kidney. Other combinations which have been described are neurofibromas and ganglioneuromas, neurofibromas and pheochromocytomas and, most commonly of all, neuroblastomas and ganglioneuromas.

In the fetus reported in this paper the tumors exhibit an inter-

mixture of cells which vary from the most primitive type of neuroblast to mature ganglion cells and from areas composed largely of ganglion cells and containing relatively few fibers to others in which ganglion cells are completely absent and only fibers and Schwann cells are visible. The tumors are much more widely disseminated and there is a much more complete involvement of the sympathetic nervous system than has previously been observed. The large fibroneuroma of the bladder is also unique. Due to the method of original fixation it is impossible to be certain whether or not there is differentiation into chromaffin cells. Although this fetus was delivered 2 months prematurely, necrosis and calcification of the tumors had already occurred.

The miliary dissemination of neuroblastic tissue throughout the liver may probably be considered a result of the same stimulus which produced neoplastic changes in the rest of the sympathetic nervous system. According to Maximow [3] (1930), collections of cells arising from sympathogonia and giving a chromaffin reaction are normally found in this organ. Most authors have considered the frequent association of hepatic tumors with adrenal neuroblastomas a result of metastases from adrenal tumor to liver. This may be a reasonable assumption in the presence of a single primary tumor. In the present case, however, stimulation of the cells normally distributed throughout the liver, simultaneous with the stimulation of the rest of the sympathetic nervous system, seems a more probable explanation of the origin of these tumor masses.

This case exemplifies most graphically the close interrelationship of ganglioneuromas, neuroblastomas and fibroneuromas and the common identity of the stimulus required for their production.

Summary

A premature stillborn fetus is described in which there was a generalized neoplasia involving all of the sympathetic ganglia, the adrenal glands and the wall of the urinary bladder. Multiple tumor foci were present in the liver. The sympathetic and spinal nerves were hypertrophied.

It exemplifies the close relationship which exists between neuroblastomas, ganglioneuromas and fibroneuromas.

Note: The authors are deeply indebted to Percival Bailey for aid and advice.

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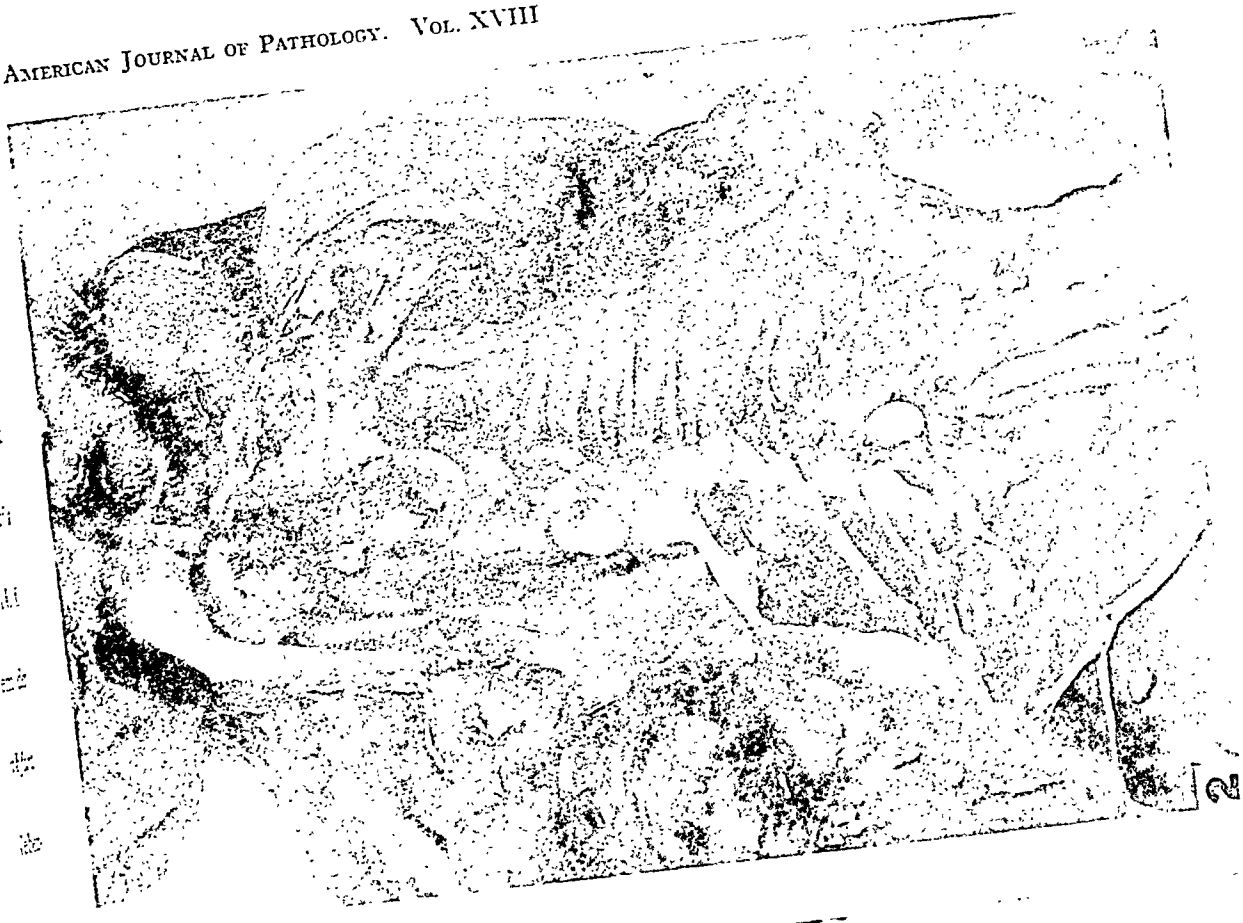
DESCRIPTION OF PLATES

PLATE 20

FIG. 1. Anterior view of the abdominal and thoracic viscera after removal of the liver. The two tumor masses in the region of the adrenal glands and the contiguous mass in the midline have forced the kidneys downward and have distorted their contour. The tumor of the bladder is visible below the other organs. A = adrenal gland visible on surface of one of tumor masses, T. B = bladder tumor. L = lungs. H = heart. K = kidneys.

FIG. 2. The thoracic and abdominal viscera have been drawn to the right revealing the numerous tumor nodules in the region of the sympathetic ganglia and along the nerve trunks. The generalized hypertrophy of nerve trunks is also well shown.

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Neuroblast

Potter and Parrish

PLATE 21

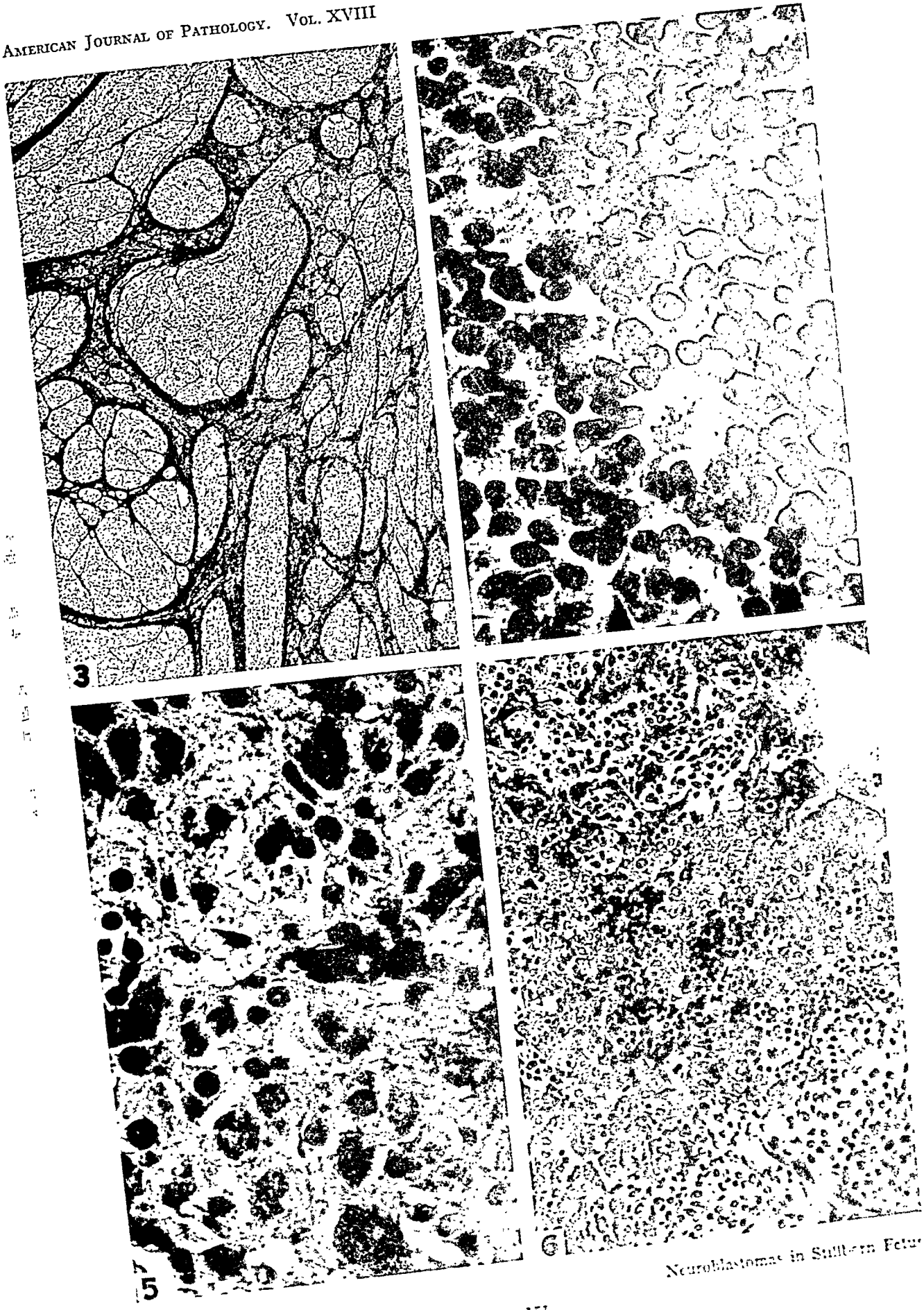
FIG. 3. Tumor from the bladder showing separation of neoplastic fibers into interlacing bundles by intervening connective tissue. No ganglionic or neuroblastic cells are present. Hematoxylin and eosin stain. × 16.

FIG. 4. Sympathicoblasts in one of the tumors of the thoracic sympathetic ganglia. Similar areas were present in all tumors except that in the urinary bladder. Hematoxylin and eosin stain. × 612.

FIG. 5. Immature ganglion cells from one of the adrenal tumors. Similar cells were found in all tumors. In the bladder they were isolated in very small areas and comprised only a small fraction of the entire tumor. Hematoxylin and eosin stain. × 612.

FIG. 6. Tumor nodules in the liver. The light-staining cells are sympathicoblasts; the darker ones are hepatic cells. The latter are interspersed with numerous normal areas of erythropoiesis. Hematoxylin and eosin stain. × 197.

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Neuroblastomas in Stillborn Fetu

ICTERUS OF THE ADULT BRAIN *

Report of a Case

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Bile pigments in the brain, to our knowledge, have never been found in adults, even in patients with severe jaundice showing marked biliary pigmentation of dura and leptomeninges. In children, however, bile pigments are observed in certain areas of the brain in the rare condition of Kernikterus. Surveys of the literature on Kernikterus are given in the recent papers of Zimmerman and Yannet,[1] and of Fitz Gerald, Greenfield and Kounine.[2] Broman,[3] in 1940, stressed the fact that bile pigment is never found in the brains of adults; the blood-brain barrier in adults is sufficiently impervious to prevent the transfer of bile from the blood vessels into the brain tissue. In view of these conclusions, we believe that observations made in the study of a recent case showing extreme jaundice are worthy of record. The brain in this case showed no changes comparable to that of Kernikterus, namely, pigmentation of circumscribed nuclear masses, but did reveal, in the cortex, several yellowish green foci containing bile pigment.

Report of Case

F. G. (C. G. H. no. 48629), a white American-born housewife, 74 years old, was admitted to the Colorado General Hospital on November 27, 1940, with the complaints of progressively deepening, painless jaundice with severe itching for 9 months, intolerance to fatty foods with associated gastric discomfort, nausea and vomiting for 2 or 3 months, burning and tingling sensations in the feet for 3 months and, for 2 months, an almost continuous desire to sleep. The patient had lost 25 pounds during the 9 months, was extremely constipated, had clay-colored stools and passed dark brownish yellow urine, with burning on urination and nocturia. Physical examination revealed an emaciated, elderly woman with a deep yellow, dry skin and marked abdominal distention. Temperature upon admission was 98.2° F.; respiration rate was 24 and pulse rate, 84 per minute; blood pressure was 120/60. Urinalysis findings were: specific gravity, 1.023; albumin, 2 plus; pus cells, 2 plus. Blood studies revealed: hemoglobin content, 11 gm. per 100 cc.; red blood cells, 3,710,000; white blood cells, 17,700 with 80 per cent polymorphonuclear leukocytes, 10 per cent lymphocytes and 1 per cent endothelials; sedimentation rate of 32 per cent in 30, and 55 per cent in 60 minutes; prothrombin time of 87 per cent of normal; icteric index of 150 and direct van den Bergh reaction. There were 19 mg. of bilirubin, 34 mg. of nonprotein nitrogen and 88 mg. of sugar per 100 cc. of blood. Roentgenogram of the chest revealed no intrapulmonic pathologic changes. Histologic examination of ascitic fluid by sectioning sediment blocked after centrifugation failed to show tumor cells. On the second hospital day, 4700 cc. of dark brown, ascitic fluid were removed from

* Received for publication, May 12, 1941.

the body, permitting the palpation of a mass in the upper portion of the abdomen. Clinical diagnosis of advanced carcinoma of the head of the pancreas was made. The clinical course was progressively downhill. Throughout most of the hospital stay the patient was stuporous. On December 6th she was transferred to a nursing home where she remained, under medical care, in a stuporous state until her death on December 26th.

Postmortem examination of the extremely jaundiced body showed a mucinous adenocarcinoma of the gallbladder. The gallbladder was small, thick-walled and contained numerous mixed pigment-cholesterol calculi. Neoplastic tissue involved the portal lymphatic system with obstruction of the common bile duct, extension along the main intrahepatic ducts, secondary cholangiectasis, suppurative choledochitis, cholangitis, associated with scattered small abscesses and focal necrosis of the liver. Other findings included suppurative cystitis and slight pyelonephritis, marked atherosclerosis of the aorta, with ulceration and calcification, and atherosclerosis of the coronary and cerebral arteries. A detailed description of the changes in the brain follows.

Gross Examination of the Brain

At necropsy the leptomeninges appeared deeply yellow-tinged and over the left occipital region presented several greenish yellow spots, 2 by 1 cm. in maximum area. In the thin walls of the main and smaller arteries at the base of the brain were numerous atheromatous plaques.

After 24 hours of formaldehyde fixation, the structures of the cortex appeared well preserved. No tumor tissue was evident macroscopically in frontal sections through the brain. In a rather large area in the right occipital lobe, conspicuous foci with a greenish discoloration were disclosed. Most of these foci were deepset in the convolutions near the sulci; the majority were limited to the cortex but a few involved the adjoining white matter. Some foci appeared as spots a few millimeters in diameter; others, as narrow stripes. In the depth of the white matter of the right cerebellar hemisphere was a small yellow focus. This was 7 by 5 by 3 mm. in size, was irregularly outlined and presented homogeneous yellow surfaces on section. Macroscopically, the brain otherwise appeared normal.

It was possible to extract a sufficient amount of the pigment with chloroform to carry out the Gmelin reaction on the crude extract; this reaction was positive. It was not possible to obtain sufficient pigment for more exact chemical identification.

Microscopic Description of the Brain

There was no evidence of metastatic neoplasm in any section of the brain tissue. The focus in the white matter of the cerebellum was surrounded by a zone of loose, edematous tissue in which the ground

substance was vacuolated and appeared reticular. The focus itself was richly vascularized: many of the vessels were dilated and some were lined by swollen endothelial cells. The tissue appeared less reticular and vacuolated than that of the surrounding zone and contained residua of broken-down myelinated fibers and scattered swollen astrocytes. It was composed chiefly of loosely arranged scavenger cells. These scavenger cells were round and somewhat variable in size; they had one or two moderately large nuclei and a granular or vacuolated cytoplasm. Several gitter cells appeared diffusely yellow-tinged, although no granular bile pigment was apparent in this focus. The area thus appeared as a focus of softening, with slight, diffuse, biliary pigmentation in some of the gitter cells but no pigmentation of the interstitial tissue. The grossly visible generalized pigmentation, not discernible microscopically, was probably the result of a diffuse imbibition with bile pigment.

The other foci were in part similar to that in the cerebellum. They showed features of softening and were surrounded by vacuolated edematous tissue. On the other hand, they contained a number of scavenger cells in which there was much more bile pigment, either diffuse or in granules of variable size with indistinct borders. In the same area, swollen astrocytes failed to display stored bile pigment. In some areas the "softening" was composed partially of a vascular syncytial tissue, the cytoplasm of which appeared clear and faintly foamy, suggestive of pseudoxanthomatous granulations. Furthermore, in other amorphous, apparently necrotic areas there were deposits of calcified material, in small granules, partially coalesced or in elongated, more or less tortuous strands, probably formed from calcified, necrotic myelinated fibers. In yet other areas, there were clusters of dense aggregates of calcified fibers and globules. Several intracortical vessels were surrounded by thick collars of calcified granules. The softened areas were located in the subcortical white matter and showed a few irregularly scattered, calcified fibers. The main calcified zone involved the deep cortical layers. The upper and middle layers of the cortex showed recent necrosis; the second layer contained many calcified nerve cells. In this area the supporting tissue showed little reaction. On application of the Nissl stain this necrotic zone clearly exhibited bile pigment. This pigment, yellowish brown and granular, was present within both scavenger and necrotic, ghostlike nerve cells, as well as in intramural cells of the vessels. In addition, the involved cells, especially the nerve cells, were diffusely pigmented. Numerous necrotic nerve cells appeared deeply eosinophilic, as is frequently evident in ischemic necrosis. In other areas in the cortex there were scattered petechiae.

Fat stain revealed the presence of lipoids in the scavenger cells and demonstrated clearly the development of such cells from progressive microglia cells. By means of a silver stain, swollen astrocytes, especially of fibrillary type, were found to be prominent in the tissue surrounding the foci. Gram-picric stain of various sections revealed no evidence of bacteria. Staining for iron was negative. Von Kossa's stain for calcium was strongly positive.

Several small arterial branches in the meninges near the foci presented mural atherosclerotic plaques, which narrowed but did not occlude the lumina. There were no thrombi. Numerous intracortical arterioles were markedly sclerosed; the thickened walls compressed the lumina. The meninges were free from inflammatory changes.

Discussion

The foci described above showed in part the features of ordinary softening as seen in arteriosclerosis. In addition they contained areas of necrosis with marked calcification. Bile pigment was present in necrotic nerve cells, scavenger cells and endothelial cells. We believe that the blood-brain barrier, which is probably located in the vascular intima (Broman,[3] Spatz [4]) became pervious to bile within these foci. It is to be concluded from the papers of these authors that trypan blue, which under normal conditions does not enter the nervous parenchyma, can do so if there is a local disturbance in the hemato-encephalic barrier. This is probably likewise true of bilirubin. A disturbance in that barrier may well be assumed to develop in the capillaries within a focus of arteriosclerotic or traumatic necrosis. As in experiments with trypan blue and in Kernikterus, necrotic tissue elements, particularly necrotic nerve cells, seem to have a certain affinity for the dye. The ensuing distribution and location of the pigment within the tissue constituents, as indicated above, are essentially consistent with the findings in Kernikterus. Normal nervous parenchyma has a lack of affinity for trypan blue (King [5]) and obviously for bile pigments.

The complete necrosis and calcification in certain areas are not readily explained. The deficient reaction of the local supporting tissues may be due, of course, to the time factor, inasmuch as the necrosis could have been of quite recent origin, with death occurring before the reactive changes set in. On the other hand, we may assume a severe toxic activity of the penetrating bile, necrotizing not only the nervous parenchyma but also the supporting tissue. A lengthy discussion of the causes of calcification in necrotic areas of the brain is beyond the scope of this paper. The local colloids possibly undergo a change so as to behave like gels; the latter display a certain affinity for calcium salts (Meyer,[6] Eaton, Camp and Love [7]).

The change was less marked in the white matter of the cerebellum than in the gray matter of the occipital lobes. This is consistent with the fact stressed by Spatz [4] that quite generally the gray matter stores vital dyes more intensely than the white matter, the former having a better vascular supply.

Although findings similar to those discussed in this paper, to our knowledge, have never been described, we are of the opinion that they may be found in patients showing a combination of extreme jaundice and cerebral arteriosclerosis.

Experimentally, it should be possible to produce obstructive jaundice of long duration and then, by traumatization of the tissue or by ligation of an arterial branch, to produce foci of brain softening. If our view is correct, such areas should show bile pigment. This would be consistent with the experiments of Broman [3] and Spatz [4] with trypan blue (tingibility of nervous tissue in foci of traumatic destruction or embolic damage).

Summary

1. Foci of cerebral softening containing bile pigment are described as found in a patient with carcinoma of the gallbladder and severe generalized jaundice.

2. The pathogenesis of this condition is discussed.

Note: We are indebted to Bernard B. Longwell, of the Department of Biochemistry, University of Colorado School of Medicine, for making the chemical examination.

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BILATERAL FUSIFORM ANEURYSMS OF THE CERVICAL PORTION OF THE INTERNAL CAROTID ARTERIES *

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During the academic year 1939–1940, there was found in the anatomical laboratory of Tulane University a cadaver with bilateral fusiform aneurysms of the cervical portions of the internal carotid arteries. The subject was a negress about 50 years old. Other pathological conditions found in the body were: primary carcinoma of the head of the pancreas, cirrhosis of the liver and uterine leiomyomata.

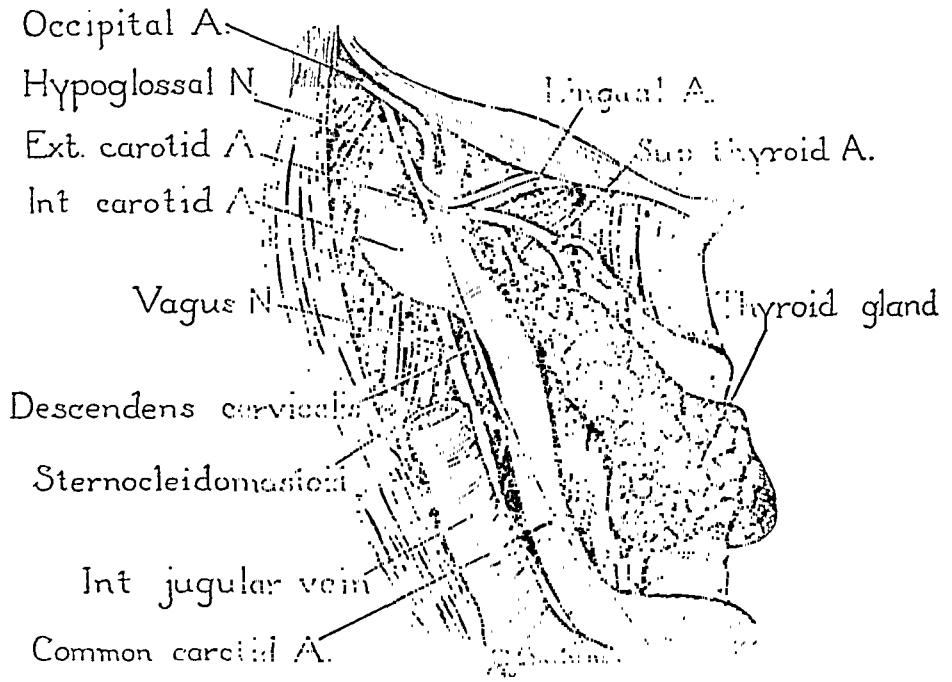
Each aneurysm was located at the beginning of an internal carotid artery and each was 19 mm. in length with internal diameters of 8 mm. The internal diameter of the vessels above the dilatations was 5 mm. Microscopical sections were made through the aneurysms and through the arteries above and below the distended portions. One set of sections was stained with hematoxylin and eosin, one set with Weigert's elastic tissue stain and another with van Gieson's stain.

MICROSCOPICAL EXAMINATION

Aneurysmal Portion of Right Carotid Artery. Sections of the dilated portion of the right carotid artery, stained with hematoxylin and eosin, showed the endothelial lining virtually destroyed, possibly as the result of autolysis. The intima generally was much thickened and very irregular in outline, some areas being thrown up into definite elevations. It presented areas of atheromatous degeneration, some of which showed lime salt deposits and other amorphous structureless material. The media showed extensive degenerative changes of the same nature, with lime salt deposits and slight increase in connective tissue. In one area the wall of the vessel was very thin, being made up of a thin layer of intima and only the outer portion of the media and adventitia. In the adventitia were noted many groups of lymphocytes and plasma cells around the blood vessels. Sections stained by van Gieson's method showed a marked increase of the connective tissue element throughout, which had largely replaced the muscular structure. In one area the inner muscular layer had entirely disappeared and the wall of this part was made up only of the intimal covering and outer medial coat. With Weigert's elastic tissue stain the normal internal elastic lamina was not demonstrable as a relatively solid, wavy cord but the elastic fibers occurred as fine fibrils throughout the wall, being most marked

* Received for publication, June 5, 1941.

in the deeper portions of the media. In one area there was a definite rupture of these fibers and the overlying structure corresponded to one of the degenerating atheromatous layers of the inner portion of the media and intima. The outer medial coat showed many of the elastic tissue fibrils running in a tangential rather than in a circumferential manner. These changes were indicative of arteriosclerotic degeneration with lime salts deposited in some areas, resulting in rupture of the intima and medial coats and permitting dilatation of the vessel with formation of a fusiform aneurysm.



TEXT-FIGURE 1. Drawing showing the fusiform aneurysm of the cervical portion of the internal carotid artery on the right side. An identical lesion was present on the left side.

Non-dilated Portion of Right Carotid Artery. Sections from this region showed some slight thickening of the intimal coat which was rather irregular but presented only small areas of atheromatous degeneration. In the media there was a slight increase of connective tissue and also in the adventitia. Perivascular infiltration was demonstrated.

Left Carotid Artery. Sections from both aneurysmal and non-dilated portions showed similar pictures to those from the right, but atheromatous changes were not as marked.

Diagnosis. While there was slight perivascular infiltration, it is not believed that syphilis was the causal factor. A histological diagnosis of arteriosclerosis (atheroma), with softening of the intimal coat,

rupture of the medial coat and fusiform aneurysms was made. The aorta showed moderate atheromatous change with no evidence of luetic involvement either grossly or microscopically.

Discussion

I have been unable to find any report of bilateral aneurysms of the cervical portion of the internal carotid arteries. Only four reports of bilateral aneurysms of the carotid arteries were found in a survey of the available literature.

Temple (1891) described bilateral aneurysms of the common carotid arteries with involvement of the innominate artery on the right side.

A short description of Gay's case (1894) of ligation of both common carotid arteries for double aneurysm follows:

> A patient, 7 years of age, noticed a small, pulsating tumor on the left side of his neck about 1 inch above the clavicle. Ten years later he noticed a tumor the size of a chestnut on the right side of the neck at the level of the upper edge of the thyroid cartilage. Nine years later, at the time of the first operation, the tumor on the left side extended from a point 1½ inches above the clavicle to the mastoid region, filling the upper part of the anterior triangle and at the same time elevating and pushing the sternomastoid muscle backwards. By this time the tumor on the right side was about 1 inch in diameter. Pressure on the common carotid arteries below the tumor stopped the pulsation in both aneurysms. Two ligatures were placed on the left common carotid artery 1½ inches above the clavicle and the artery divided between the ligatures. Ten months later the right common carotid artery was ligated and divided about 2 inches above the clavicle. Four months after the second operation a small pulsating tumor appeared in the center of the sac on the left side. This secondary aneurysm increased in size very slowly. Nineteen months after the second operation the patient was perfectly well except for the small secondary aneurysm which gave him no trouble.

Friedrich (1934) described a patient with exophthalmos and other symptoms which indicated an hypophyseal tumor. The autopsy disclosed an aneurysm of the cavernous portion of each internal carotid artery.

Bozzoli (1937) described bilateral aneurysms of the cavernous portions of the internal carotid arteries. Pressure of the dilatations on the pituitary gland caused an ocular syndrome and acromegalic signs.

Summary

An example of bilateral fusiform aneurysms of the cervical portions of the internal carotid arteries is described.

Note: The microscopical sections were examined by Dr. John A. Lanford, Department of Pathology and Bacteriology, Tulane University.

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TRYPAN-BLUE VITAL STAINING IN STUDIES OF VIRUS LESIONS ON CHORIO-ALLANTOIC MEMBRANES *

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In the cultivation of filtrable viruses on the chick chorio-allantois, the cellular proliferations produced by the virus on the membrane appear characteristically as discrete, semi-opaque gray foci. In the case of some viruses, such as variola and vaccinia, for example, these foci are relatively large and easily seen. On the other hand, with certain viruses, among which may be numbered encephalitis, influenza, yellow fever and lymphocytic choriomeningitis, the lesions produced are so small that all investigators have experienced difficulty in recognizing them. This is especially true under certain conditions, and when the foci are few in number. Obviously, lesions must reach a certain size to be visible even with a hand lens, and since they often measure less than 0.5 mm. when fully developed, scanty, scattered foci cannot be recognized. In this paper there is described a method for making these small focal lesions visible and readily recognizable by staining them with trypan-blue, and for making permanent mounts of such stained membranes for later, more careful examination.

Trypan-blue, after intravenous injection, has the property of being taken up and flocculated by certain living cells, notably reticulo-endothelium, and to a certain degree by other connective tissue elements. It stains only living cells, and does not produce a diffuse coloration of all tissue elements. So far as we are aware, the use of the dye for differentiation and demonstration of proliferative virus foci on egg membranes has not been described previously.

Our earlier observations on the effect of injecting trypan-blue into a vein of the chorio-allantois showed that almost all of the pigment was retained in the embryo, which became blue, but that the membrane itself remained unstained. However, if the trypan-blue solution was applied directly to the membrane containing virus lesions and the egg reincubated for a short time, it was found that the areas of focal proliferation became blue, while the remainder of the membrane was only faintly stained. In normal membranes of uninoculated eggs treated in the same manner, the cells take up only minute amounts of the stain. The membranes are pale blue and without focal intensifica-

* Received for publication, May 29, 1941.

tion. Virus-infected membranes, after being stained, removed from the egg and washed free of the excess stain, can be fixed flat in a solution of formaldehyde and mounted in glycerol jelly under a coverglass. This serves to "clear" the preparation and brings out more distinctly the differential staining of the focal lesions. Such mounted specimens are permanent and can be studied with the higher powers of the microscope. Details of the staining and mounting technic follow.

Method

When a membrane is ready for examination, 1 cc. of a 0.5 per cent aqueous suspension of trypan-blue is placed directly upon the membrane through the window in the shell. Good general distribution of the stain may be obtained by gentle rotation of the egg. More recently, however, we have found it more satisfactory to apply the stain by means of an atomizer (DeVilbiss no. 15), of which the tip may be directed downward. In this case no rotation is necessary. After staining, the shell window is closed and the egg placed in the incubator for from 10 to 30 minutes, depending on the age and extent of the lesions. More extensive lesions require a shorter time to take up the stain, and may become too intensely colored for satisfactory examination if the stain is left on too long.

The membrane is removed in the usual manner, washed gently in physiological saline solution to remove the excess of trypan-blue, and fixed flat in a 4 per cent solution of formaldehyde for a few minutes. After draining away the excess fluid, it is placed on a glass slide, 2 by 2.5 inches, and mounted in a glycerol-gelatin mixture consisting of 50 per cent glycerol, 5 per cent gelatin and 1 per cent phenol. The gelatin should be soaked in water for about 2 hours before adding the glycerol and phenol, then heated gently for a few minutes while stirring. For mounting the membrane, the glycerol jelly is melted and, while still warm (about 70° C.), is dropped on the membrane until it is well covered. The slide is flamed gently, but not enough to produce bubbles, and then mounted with a warmed coverslip. Gentle pressure is applied until the jelly has begun to set. When fully hardened the excess jelly is removed and the edges sealed. For this purpose clear Duco cement thinned with acetone is very satisfactory.

Discussion

The method outlined has been used routinely during the past year on several thousand membranes, and has been of considerable value in the recognition of virus lesions which could not have been identified without its use. While most of the observations have been made with St. Louis encephalitis virus, we have found that this method shows equally well the lesions of Japanese encephalitis, lymphocytic choriomeningitis, influenza, yellow fever, herpes simplex, vaccinia and variola. Normal membranes, and those to which clear broth or sterile serum had been added 1 to 2 days previously, when stained by trypan-blue and mounted, show none of the focal staining characteristic of virus lesions. Microscopic examination of these membranes reveals faintly stained ectodermal cells, occurring in small groups or sheets. Cells around areas of trauma may also appear stained. After a little

practice, however, no difficulty is experienced in identifying virus foci from non-specific or traumatic areas.

The recognition of lesions has been particularly helpful in studies of St. Louis encephalitis in which the foci produced are small. By the method described it is often possible to recognize the stained lesion as early as 18 hours after inoculation, and always after 24 hours. Even when the lesions are few and scattered, as with inoculation of 0.1 cc. of 10^{-7} dilution of the virus, they can be identified regularly as virus foci. Influenza virus foci, which are even smaller than those of St. Louis encephalitis, are clearly differentiated and recognized by the trypan-blue stain. In addition, the method has been valuable in the titration of virus dilutions, since scanty, scattered lesions are readily visible; and also in the study of tests of immune serum neutralization, in which the absence of foci, or the counting of the lesions, are important criteria.

Under low magnification the lesion appears as a discrete focal accumulation of proliferating cells stained blue, and in sharp contrast with the surrounding membrane. In older lesions some necrosis may be seen in the center, and some infiltration of inflammatory cells at the periphery. With higher magnification the proliferating cells contain many fine blue granules. Mounts of entire membranes are not very satisfactory for detailed cytological study because of their thickness, but after imbedding in paraffin, cross sections show the characteristic focal proliferation of ectodermal cells containing trypan-blue granules. As yet we have made no careful study of the appearance of these lesions in cross section.

The use of other stains, such as janus green and vital red, has been rather unsatisfactory since they produce a more general staining of the membrane, and differentiation of the foci is poor. While trypan-red is similar to trypan-blue, the chorio-allantois takes up the red stain more diffusely and identification of individual lesions is more difficult.

The permanent mounting of membranes bearing virus lesions has numerous advantages, facility is acquired readily and the mounts may be made routinely by technicians. The membranes may be thus preserved and filed, and the lesions studied by several observers at any later time. Actual membranes may be projected directly on a lantern screen for demonstration.

Summary

A method of staining virus foci on chick chorio-allantoic membranes by trypan-blue is described, with directions for clearing and mounting such membranes for permanent record and study. Its use facili-

tates greatly the recognition and enumeration of the lesions in the study of viruses and virus immunity in the developing egg.

NOTE: We wish to express our appreciation to Margaret G. Smith of the Department of Pathology of the Washington University School of Medicine, and to Edwin H. Lennette of the International Health Division of the Rockefeller Foundation, for furnishing viruses used in this study. Antoinette Wagenfuehr assisted in the technical procedures.

DESCRIPTION OF PLATE

PLATE 22

FIG. 1. Vaccinia (48 hours, dilution 10^{-6}). The lesions appear as large, discrete foci, standing out in sharp contrast to the normal membrane. While the large pocks of vaccinia may be seen readily on unstained membranes, this stained preparation emphasizes the striking differential staining reactions of virus-infected cells of the chorio-allantois. × 3.

FIG. 2. St. Louis encephalitis (72 hours, dilution 10^{-3}). Here is seen a somewhat diffuse, faint staining of the membrane on which more deeply stained focal areas of cellular proliferation stand out prominently. × 3.

FIG. 3. Herpes (72 hours, dilution 10^{-1}). Very numerous, deeply stained foci, for the most part discrete but confluent in certain areas. × 3.

FIG. 4. Herpes (72 hours, dilution 10^{-1}). An identical sister membrane unstained for comparison, but under slightly greater magnification. The herpetic foci appear merely as somewhat indefinite, hazy, poorly defined opacities. × 3.5.

FIG. 5. St. Louis encephalitis (same as in Fig. 2). Higher power of Figure 2 showing the sharp differentiation of the virus foci from the faintly stained membrane. × 35.

FIG. 6. Herpes. Same membrane as Figure 3. × 35.



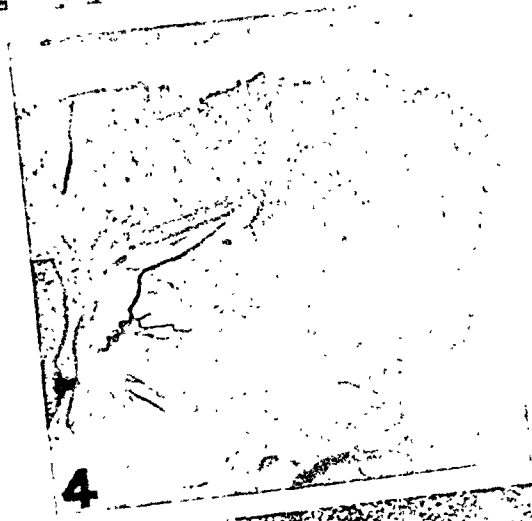
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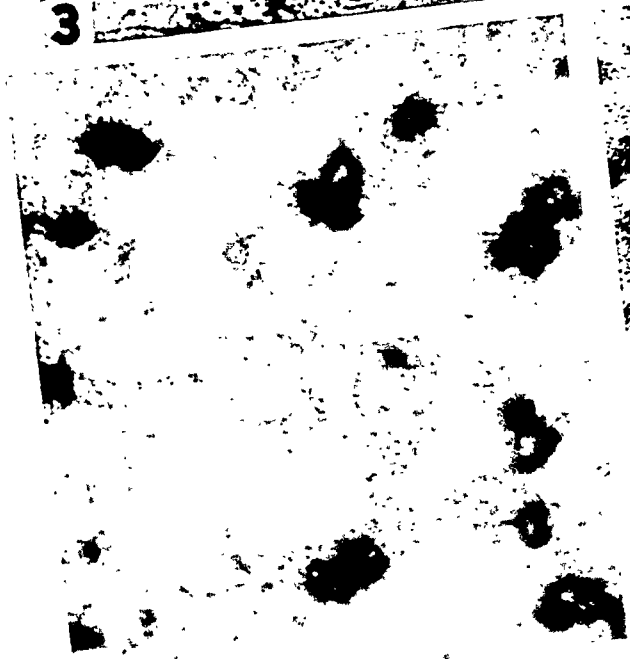
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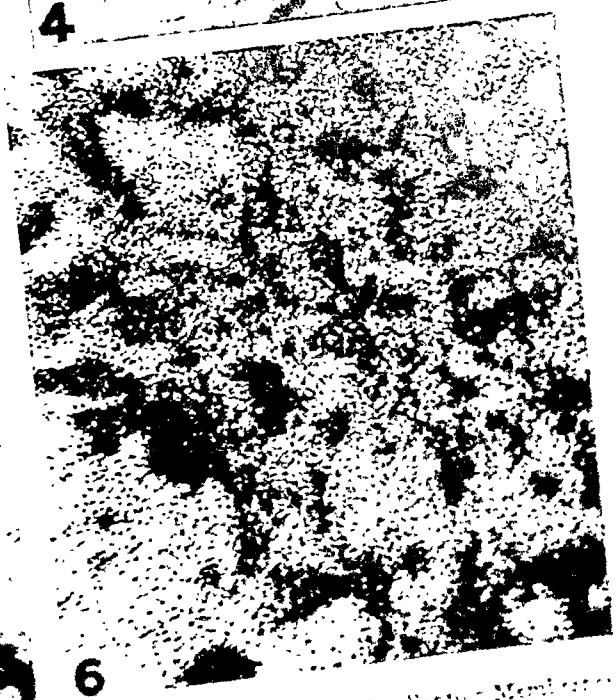
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Trypan-Blue Staining of Chorioallantoic Membranes

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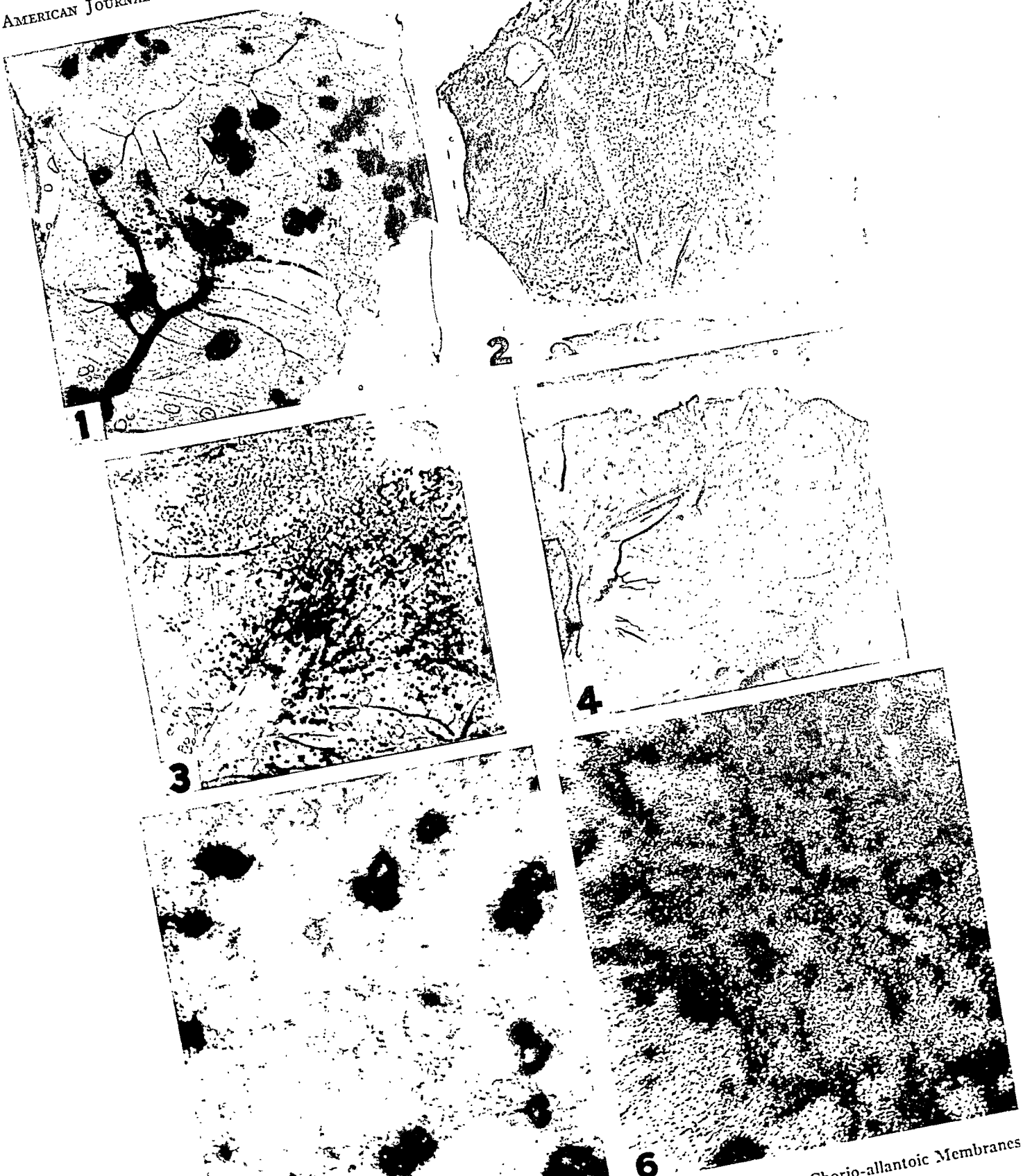
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AMERICAN JOURNAL OF PATHOLOGY. VOL. XVIII



Trypan-Blue Staining of Chorio-allantoic Membranes

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THE AMERICAN JOURNAL OF PATHOLOGY

VOLUME XVIII MARCH, 1942 NUMBER 2

MUSCULAR DYSTROPHY IN MICE ON VITAMIN E-DEFICIENT DIET*

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It has become evident during recent years that vitamin E is a necessary factor for the preservation of the integrity of skeletal muscle. This has been satisfactorily demonstrated for a number of species: guinea pigs,[1-4] rabbits,[1, 5-7] goats and sheep,[8] rats,[9-11] the tree kangaroo,[12] ducklings[13] and, probably, the dog.[14, 15] Muscular lesions may, under conditions not yet accurately defined, occur also in young chicks upon diets low in vitamin E.[16, 17]

A comprehensive study of the effects of vitamin E deficiency in mice, carried out by Marianne Goettsch,† has provided material for a report on the pathology in this species. Little has been previously written on the effects of vitamin E lack in mice, and we have seen no reference to lesions of the skeletal muscles such as will be described in this paper. Indeed, the literature affords very little information as to the requirements of mice for vitamin E. Beard,[18] in 1926, demonstrated that this factor was needed for the successful completion of pregnancy and Bryan and Mason [19] have recently shown that, unlike the rat, the male mouse fails to develop testicular degeneration and sterility on diets low in vitamin E—an observation in complete agreement with our experience. These authors also state that "the neuromuscular disorder so frequently seen during late stages of lactation in the progeny of female rats possessing a critically low storage of vitamin E was not observed in any of our suckling mice."

MATERIAL AND METHODS

The mice were the offspring of mothers maintained through 2 to 7 generations on the following vitamin E-deficient diet:

* Received for publication, June 23, 1941.

† This will be published elsewhere.

Diet 1

Casein (technical)	320
Cornstarch	400
Salt mixture	40
Yeast (Fleischmann's brewer's)	100
Lard	220
Cod liver oil	20

During the lactation period, the amount of yeast was doubled. The mothers were given a single dose of alpha-tocopherol or of wheat-germ oil at the beginning of pregnancy. Details of dosage necessary to insure fertility will be published elsewhere by Dr. Goettsch.

Tissues from 293 mice were available for histologic study. Animals which died or were killed on the first or second day after birth were fixed *in toto* for several days in Bouin's fluid. In somewhat older mice, the entire limbs were fixed in Zenker's fluid, and from adult animals, various samples of muscle and viscera were examined individually. Complete study of the central nervous system, after fixation in a 4 per cent solution of formaldehyde, was carried out on a few young and old animals. The following methods were applied: the gallocyanin stain for ganglion cells; Weil's stain for myelin sheaths; Bodian's stain for axons; phosphotungstic acid hematoxylin for possible glial reactions. No systematic study was made of the endocrine organs.

Unlike young rats, which in most cases betray the onset of muscle lesions by dragging their hind limbs and clenching their paws, mice exhibit no recognizable symptoms even when lesions of considerable severity are found. We have not observed a single instance of frank paralysis, and have been unable to predict the lesions while animals were alive. Nevertheless, gross changes in the appearance of the muscle could be detected in some of the more severely affected animals, in the form of yellowish gray streaks.

Microscopic Changes

The material has been arranged on the basis of the age of the mice, since the lesions differ to a certain extent according to age.

Of 22 mice obtained on the first day after birth, 14 were found dead, 8 were killed. However, no animals are included in which the state of preservation, as judged by nuclear staining, was bad.

Edema of the subcutaneous and intramuscular connective tissue, of varying severity, was present in 12 mice, including 3 that were killed, so that it cannot have been due to autolytic change (Figs. 1 and 2). In a few cases, it was accompanied by capillary hemorrhages. *Hyaline*

necrosis of the muscle fibers, sometimes accompanied by pyknosis and fragmentation of the nuclei, was recorded in 7 cases (Fig. 3). The fibers were often of unequal caliber and widely separated by edematous tissue infiltrated with small and large mononuclear cells. In a few instances, polymorphonuclear leukocytes were accumulated about the necrotic fibers. The presence of an inflammatory reaction is definite proof that the degeneration of the fibers is not an artefact or due to trauma or autolysis.

The immature appearance of the muscle fibers, associated with abnormally active blood formation in the liver, suggests that the lesions may have developed toward the end of fetal life rather than *post partum*. However, it is difficult to be certain of this.

The young were from the second to the sixth generation maintained on the vitamin E-deficient diet throughout life. It is not possible to say with assurance that the severity and frequency of the early lesions increases in successive generations, because the number of mice in each generation is too small to make percentages significant. However, in the second and third generations, only 4 of 11, or 36.4 per cent, were affected, whereas in the fourth, fifth and sixth generations, 15 of 27 examined, or 55 per cent, showed lesions—a difference which is perhaps suggestive. This calculation includes only mice dying or killed during the first 5 days.

In this group of 1-day-old mice, edema of the subcutaneous and intramuscular tissue was a more striking feature than necrosis of the muscle fibers. This finding is perhaps analogous to the condition described by Pappenheimer, Goettsch and Jungherr,[16] and subsequently by Dam and Glavind [20] and by Bird and Culton [21] as occurring occasionally in young chicks maintained on a vitamin E-deficient diet. The latter authors have shown that the edema is prevented by wheat-germ oil and by alpha-tocopherol, without other alteration of the diet, so that it may justly be regarded as a manifestation of vitamin E deficiency. Glavind[17] has recently reported the occurrence of muscle degeneration in these edematous chicks; Pappenheimer, Goettsch and Jungherr had also noted lesions of the muscles, and a reëxamination of their material shows that lesions were present in a large proportion of their edematous chicks.

Edema of the muscles is also present during the early stages of the muscular dystrophy of young rats, which occurs at the end of the lactational period. Here, however, it appears to be part of the very violent inflammatory reaction called forth by the sudden necrosis of the fibers and it is often fibrinous in character.

In the next group of 16 mice which died or were killed during the

period from the second to the fifth days inclusive, edema was no longer a feature, and indeed was noted in but one case. Five mice of this group showed hyaline necrosis of the muscle fibers. In none were the lesions very widespread or accompanied by a marked inflammatory reaction or by calcification of the necrotic fibers. In one animal, killed on the fourth day, there was loss of fibers with replacement by young fibrous tissue.

In the third group, comprising 175 mice sacrificed between the 6th and 15th days, the incidence of muscular lesions was much less. Only 17, or 9.7 per cent, showed hyaline necrosis with segmentation into fragments. With one exception, an animal dying on the sixth day, the affected mice were all between the ages of 10 and 15 days. The inflammatory reaction was sometimes slight, sometimes intense (Fig. 4). Calcification of the necrotic fibers was seen in most instances and seemed to follow almost immediately upon the death of the fibers. Often the longitudinal striations were discernible even in the completely calcified muscle fragments.

The fourth group consisted of 32 mice ranging in age from 16 to 35 days. These showed the highest incidence of muscular lesions, namely, 19, or 59 per cent. This age period includes the end of lactation, at which time the young of vitamin E-depleted rats commonly show the most severe dystrophy. The lesions were essentially the same as those occurring in rats, save that the calcification of the necrotic fibers was present in almost every case (Figs. 5 and 6). In one litter killed on the 25th day, only calcified fibers remained; no fresh necrosis or cellular reaction was to be seen (Fig. 7). This was interpreted as complete healing of an earlier previous lesion.

The last group comprised 48 mice killed or dying at various ages from 35 to 439 days. Three of the older rats succumbed to suppurative bronchiectasis and pneumonia. The others showed no abnormal findings apart from the muscular lesions noted on histologic examination. These consisted of isolated necrotic fibers, usually but not always calcified, encapsulated by multinuclear cells or histiocytes (Fig. 8). The great mass of muscle had a normal structure, and it seems evident that the necrotic fibers represent the remains of lesions incurred in early life. In several cases, the calcified fibers were surrounded by adipose tissue, which had obviously replaced degenerated fibers.

The disease thus seems to run its course during the first month of extra-uterine life; and it is not progressive. In this it is comparable to the muscular dystrophy of young rats, which, if the animals survive, undergoes spontaneous cure, even though vitamin E is withheld. The muscular dystrophy of rabbits and guinea pigs, on the other hand, is

a progressive disease often continuing into adult life, and running a chronic course.

A summary of these observations is given in Table I. No alterations of significance were found in the brain, cord or peripheral nerves, nor in the bones or in any of the viscera examined. As in other species, the smooth muscle and myocardium were not affected. In the day-old mice with edema, hematopoiesis in the liver seemed unusually active, but there was a wide individual variation, and we are inclined to regard this as merely an index of delayed development. This is perhaps also true of the muscle fibers, which were of unusually small caliber, had centrally placed nuclei and abundant undifferentiated sarcoplasm.

TABLE I

Age (days)	No. of mice	Lesions	Percentage with lesion
1	22	13	59
2–5	16	5	31
6–15	175	17	9.7
16–35	32	19	59
36–439	48	5	10
Total	293	59	20

As was stated, we can fully confirm the report of Bryan and Mason [19] as to the ineffectiveness of vitamin E deprivation in causing testicular degeneration. Thus five male mice from 221 to 439 days old showed mobile sperm, active spermatogenesis in sections of the testes and fertility on mating.

CONCLUSIONS

1. Female mice maintained on a vitamin E-low diet were given a single dose of wheat-germ oil or of alpha-tocopherol at the beginning of pregnancy to insure the birth of living young.

2. Examination of the skeletal muscles of their offspring showed necrosis of the fibers in about 20 per cent of 293 mice examined.

3. Mice dying or killed on the first day had edema of the subcutaneous and intramuscular tissues; in about one-third of the cases there was hyaline necrosis of the muscle fibers.

4. The incidence of muscular lesions was highest (59 per cent) in a group sacrificed or dying between the ages of 16 and 35 days. There was early calcification of necrotic fibers and active regeneration.

5. Adult mice occasionally showed scattered hyaline or calcified fibers remaining from early lesions, but no progressive dystrophy of the muscles.

6. No lesions of significance were found in the central nervous sys-

tem, or in other organs or tissues. In mice, spermatogenesis was active on the vitamin E-deficient diet up to 439 days.

NOTE: We wish to acknowledge our indebtedness to Claudia Schogoleff for her assistance in carrying out the experiments and to express our thanks to Dr. Abner Wolf for reviewing the sections of the central nervous system. Dr. R. D. Shaner, of Hoffmann-LaRoche, Inc., very kindly supplied the alpha-tocopherol used in this work.

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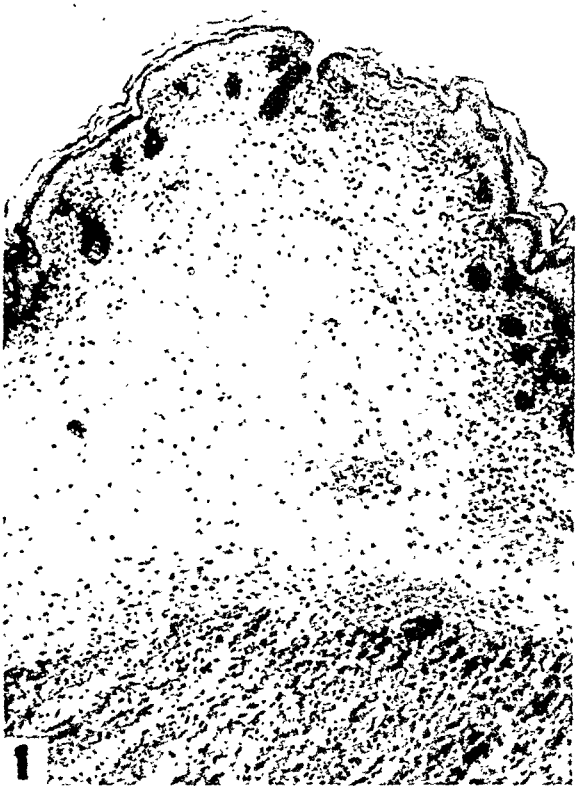
DESCRIPTION OF PLATES

PLATE 23

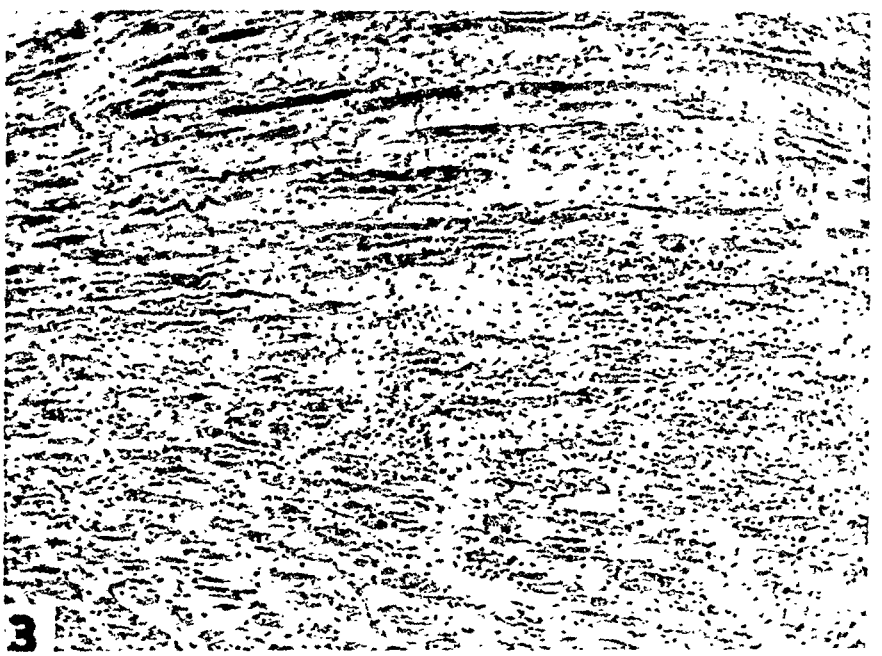
FIG. 1. Mouse no. 122a. Died on first day. Marked subcutaneous and intramuscular edema. Swollen hyaline fibers with pyknotic nuclei. × 95.

FIG. 2. Mouse no. 84a. Killed on third day. Normal skin and muscle. × 95.

FIG. 3. Mouse no. 122a. Died on first day. Edema; separation and hyaline necrosis of fibers; capillary hemorrhages. Cellular reaction. × 110.





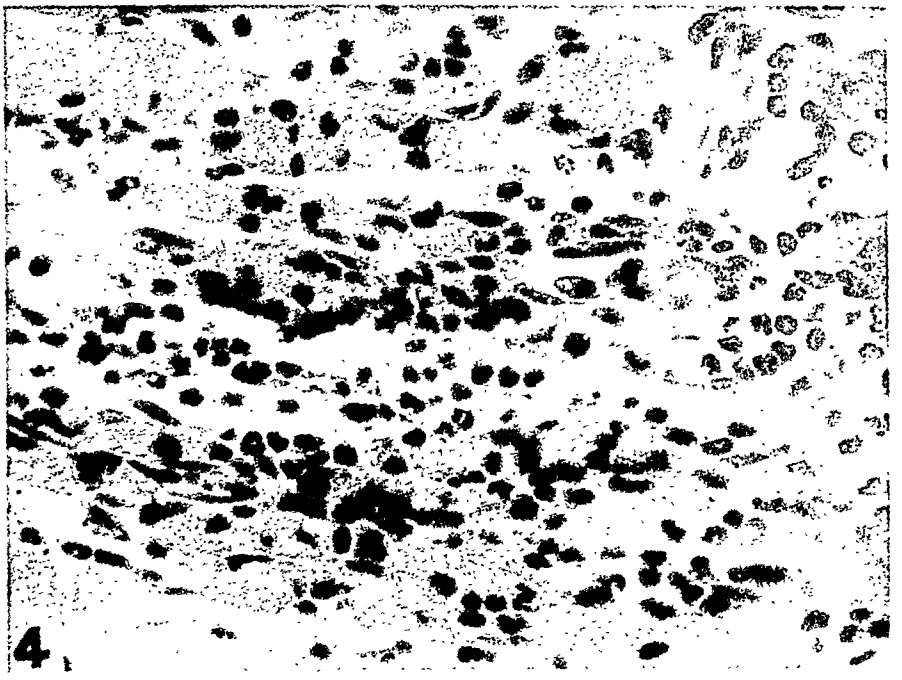


Pappenheimer Muscular Dystrophy and Vitamin E Deficiency

PLATE 24

FIG. 4. Mouse no. 277a. Killed on eleventh day. Hyaline necrosis of fibers with polymorphonuclear reaction. × 460.

FIG. 5. Mouse no. 110b. Killed on 16th day. Extreme necrosis of fibers with calcification. × 110.





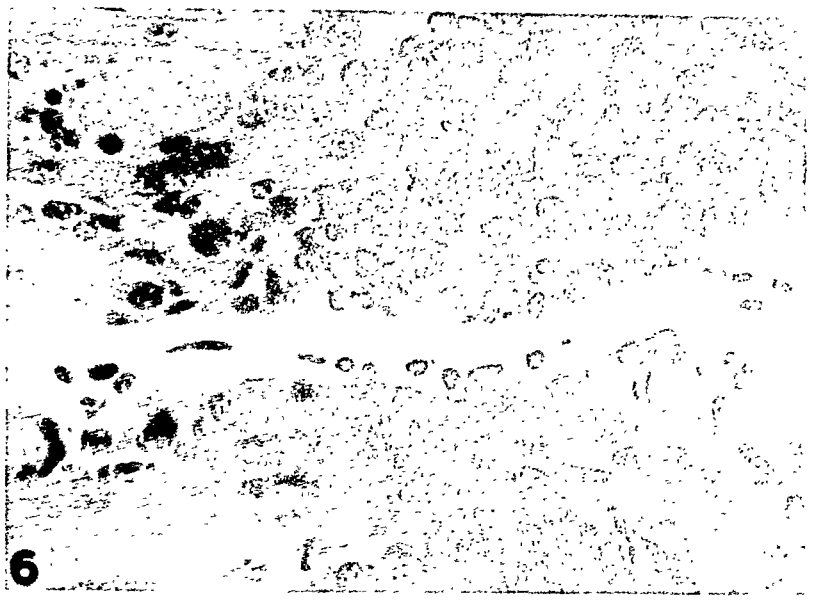
Pappenheimer Muscular Dystrophy and Vitamin E Deficiency

PLATE 25

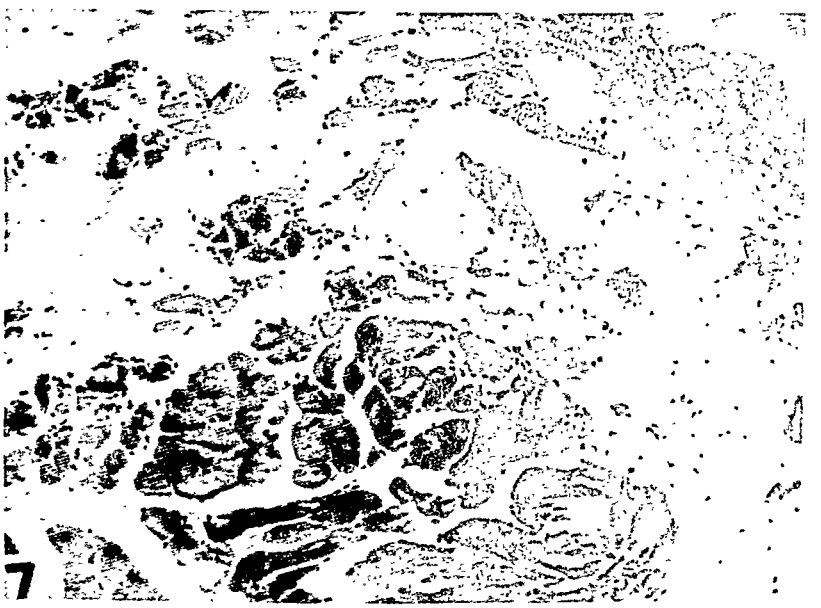
FIG. 6. Mouse no. 122d. Killed on 24th day. Necrosis of fibers; activation of myocytes; early regeneration. × 414.

FIG. 7. Mouse no. 192e. Killed on 25th day. Calcified fibers surrounded by adipose tissue. No reaction. Healed lesions. × 99.

FIG. 8. Mouse no. 310. Killed on 123rd day. Old calcified fibers. × 414.



6



7



8

THE GLOMUS TUMOR *

Investigation of Its Distribution and Behavior, and the Identity of Its "Epithelioid" Cell

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The glomus tumor is reputed to be an enlarged caricature of the highly specialized glomic arteriovenous anastomoses which have been found only in certain parts of the hands and feet at the cutaneous-subcutaneous junction. Is it really true, as reported, that glomus tumors occur elsewhere in the body, and if so how can this be explained? Are glomus tumors always small encapsulated neoplasms or do they ever display infiltrative growth or metastasize? What is the nature of the "epithelioid" cells which are so characteristic of the glomus tumors? Certain observations have come to our attention which throw some light on these phases of glomus tumors. We propose to discuss them in the order given.

Distribution of Glomus Tumors

In 1935, Masson published a monograph entitled "Les glomus cutanés de l'Homme." In this he described the normal glomuses of the hands and feet and stated that his personal experience with glomus tumors included 27 cases: 14 in the fingers and hands, none in the feet and 13 in other parts of the upper and lower extremities. All of these involved cutaneous and subcutaneous tissues. He stated that their occurrence in places where normal glomuses have never been found might be explained in one of two ways: either glomuses exist in these areas but are so widely scattered that the chance of biopsies has never permitted their discovery, or else such glomus tumors come from heterotopic glomuses.

A survey of 240 cases reported in 89 different publications, including 33 personally observed cases, indicates that the distribution is much wider than this and that the tumors can arise in other zones beside the cutaneous-subcutaneous junction. Three tumors have been found in the face. Two of these were reported by Butz (1940) and one in the eyelid was studied in this laboratory and reported by Kirby (1941). Grauer and Burt (1939) found two on the penis; Gumpel (1939), one in the axilla; Roger and Alliez (1938), one on the lateral thorax in the region of the twelfth rib; Touraine, Solente and Renault (1936) and

* Received for publication, July 21, 1941.

Schulte and Isselstein (1935), several tumors scattered over the trunk; Lendrum and Mackey (1939) reported one on the buttocks (we also have observed one in this region), and Kirchberg (1936) reported one in the neck and another from the back. Fernández and Monserrat (1931) and Sannicandro (1936) reported finding them in the auricle but there is doubt whether these are true glomus tumors. Kolaczek (1875), Buzzi (1887), v. Hleb-Koszańska (1904) and Kofler (1936) have each reported a glomus tumor of Luschka's coccygeal glomus.

The evidence that glomus tumors can arise away from the cutaneous-subcutaneous junction and from Luschka's glomus is scanty but convincing. André-Thomas (1933) described a patient with two glomus tumors: one within the substance of the vastus internus muscle and the other in the region of the internal condyle of the femur adherent to the aponeurosis of the crureus muscle where it fuses with the vastus internus. These were accepted as glomus tumors by Oberling and Roussy and mentioned in 1935 by Masson. Hoffmann and Ghormley (1941) found a characteristic glomus tumor within the capsule of the knee joint. They were kind enough to permit us to examine a section and there can be no doubt about the diagnosis. The multiple tumors described by Bergstrand (1937) included one near the sheath of the peroneal tendon, another subfascial near the lateral malleolus, a third behind the malleolus in a hollow in the talus. Swenson's (1940) patient had a glomus tumor of the palmar surface of the left middle finger which lay deep to the fat pad almost on the periosteum. In Kulenkampff and Heilmann's (1940) case the glomus tumor thickened the wall of a vein which ran along the abductor pollicis longus and brevis muscles. We have studied a similar case in which the tumor surrounded a subcutaneous vein situated on the dorsal aspect of the base of the thumb. Iglesias de la Torre, Gomez-Camejo and Palacios (1939) have described a tumor of characteristic symptoms and morphology which lay buried within the terminal phalanx without any involvement of the tissues outside of it. The cases of Durante and Lemeland (1928) in the uterus and of Kirshbaum and Teitelman (1939) in the omentum are too questionable to be considered here.

The evidence just cited seems sufficient to warrant the belief that glomus tumors can develop not only in the junction of skin and subcutaneous tissue in regions of the body where no glomuses have ever been observed but they can develop also in deeper structures.

Infiltrative Growth, Recurrence and Metastasis

Almost all observers of glomus tumors have been impressed by the fact that they are simply enlarged, encapsulated caricatures of the normal glomus and that they are easily removed and do not recur.

Nevertheless, locally recurring tumors have been reported by Lewis and Geschickter (1935) in the forearm, by Meyers (1939) in the thigh and by Kirby (1941) in the eyelid, and we have studied three other recurrent cases beside that of Kirby—one in the knee region, a subungual tumor of the finger and the third in the distal anterior closed space of a finger. It need not be supposed that these recurrent cases prove infiltrative growth, since they may be explained either as incomplete excisions or as the formation of new glomus tumors. It is now well established that individual patients may have multiple glomus tumors: Adair (1934), André-Thomas (1933), Bergstrand (1937), Davies, Hellier and Klaber (1939), Hval and Melsom (1936), Schulte and Isselstein (1935), Stout (1935), Touraine, Solente and Renault (1936), Weidman and Wise (1937).

So far as we are aware, there have been reports of only two tumors which have received the label of malignant glomus tumor. One was described by Kirshbaum and Teitelman (1939). It arose seemingly in the omentum, invaded the stomach and metastasized to the liver, and because it consisted of rounded cells collected into many layers about vascular endothelial-lined tubes, was called by the late R. H. Jaffé a malignant glomus tumor. The other case, a tumor of the pectoral region, was reported by Soiland (1937), and the reasons for calling it a glomus tumor do not appear in the text or illustrations. It seems to us that neither of these cases can be accorded unqualified acceptance as a glomus tumor.

Report of Case. Through the kindness of Dr. C. Zent Garber, pathologist of the New York Orthopaedic Hospital, we have had the privilege of studying a progressively infiltrating vascular tumor which we have good reason to believe is an authentic glomus tumor.

The patient was an Italian male. In December, 1927, at the age of 14, he began to have sharp pains below the right internal malleolus on weight-bearing, and also some tenderness. When examined a year later he had a slight foot drop, the foot was cool and damp and there was a vague soft tissue mass internal to the os calcis in the region of the calcaneo-astragaloid joint. The mass extended downward during the next 8 months and an abnormal pulsation was felt over it, with a rusty discoloration of the skin. A tumor measuring 5 by 2.5 cm., of indefinite outlines, was excised in August, 1929. It lay caudad to the posterior tibial vessels and nerves and did not appear grossly vascular. There was evidence of recurrence 2½ years later. The patient refused further treatment and was not seen until November 7, 1940, more than 11 years after the first operation, when he returned because of suddenly developed pain and tenderness in the tumor. Examination now showed extension of the tumor anterior to the malleolus onto the dorsum of the foot and also upward posterior to the ankle. There was marked atrophy of the leg and foot muscles, the foot was cold and blanched, it hung at 110° equinus and motion was possible only from 110° to 95°. A second attempt was made to excise the tumor on December 2, 1940. It extended widely in the tissues covering the medial and dorsal aspects of the tarsal bones, the medial malleolus and below it and behind the ankle joint. It was adherent to the posterior tibial nerve and artery and the

dorsalis pedis artery. As this operation obviously failed to remove all of the neoplastic tissue and the remaining tumor continued to grow, causing ulceration, an amputation was performed on February 5, 1941, through the upper third of the leg. The tumor now had extended 3 cm. above the insertion of the Achilles tendon which was surrounded by it.

Microscopically this tumor proved to be a vascular neoplasm of an infiltrative type. At its advancing margin the vessels which sometimes contained erythrocytes were simple tubes of endothelium often surrounded by collagen sheaths (Fig. 1A). Very quickly, however, rounded cells appeared in the vessel wall attached to the outer aspect of the tube. These surrounded the vessel in layers, as a rule from one to five cells thick (Figs. 1A and 1B). The cells in every way resembled the epithelioid cells of the glomus tumor. They were rounded with a centrally placed hyperchromatic nucleus, often a clear zone about the nucleus, and many of the cells were separated from their neighbors by delicate reticulin fibers. Occasionally the tumor cells with their nuclei were elongated and sausage-shaped, but this was uncommon. The sheathed vessels appeared scattered throughout the invaded tissues, which usually were not destroyed by them. At infrequent intervals tumor nodules were formed consisting of a tightly packed mass of sheathed vessels so arranged that the epithelioid cells of one vessel were in continuity with those of its neighbors and the nodule appeared like a solid mass of epithelioid cells with endothelial tubes scattered through it (Fig. 1C). The tumor vessels hardly ever formed straight tubes but twisted and turned sharply so that they were usually seen in cross and tangential sections. The tumor vessels were in continuity with arteries and veins but in none of the sections examined could any smooth muscle be demonstrated in the walls of tumor vessels nor could myofibrils be found in the epithelioid cells. None of the tissue was fixed in chloral hydrate so that no satisfactory neurite stains were obtained and we can furnish no information about this feature.

On histological grounds alone there is good reason for believing that this is an infiltrating glomus tumor. But further evidence in favor of this interpretation has been obtained from the study of tissue cultures made from this tumor and their comparison with cultures previously obtained from two ordinary glomus tumors. The study of these cultures has revealed some interesting facts about the "epithelioid" cells which we believe throw some light on their nature.

Characteristics of the Glomus Tumor in Vitro

Infiltrated Area

Cultures made from the external, infiltrative zone of this tumor, which is composed almost entirely of meandering capillaries, produce an outgrowth of fibroblast-like cells. Often these form a membranous

sheet which, when treated with silver nitrate, shows sharply stained cement borders around cells in mosaic arrangement. The nuclei of these cells are greatly flattened, as is frequently the case with endothelium, rather poor in chromatin, and surrounded by a few perinuclear granules and a wide and greatly attenuated ectoplasmic zone (Fig. 10). Such a membrane usually grows over the surface of the clot, and often covers a stratum of spindle-shaped or fan-shaped fibroblasts which push their way through the clot and do not take the silver nitrate stain.

Tumor Nodules

When a tumor nodule composed mainly of epithelioid cells of the glomus type is cultivated *in vitro*, the so-called epithelioid cells show themselves to be far from epithelioid. Their habit is discrete, their cell-body is not voluminous, and they have many ramose processes. The progress and cellular constituents of such a culture handled by the Maximow method, are as follows:

1. During the first 24 hours macrophages and small lymphocytes make their appearance, as in cultures from the infiltrative zone. Some mast cells are present in the explant.

2. Within 48 hours there is to be seen at the edge of the explant a corona of fine hairlike pseudopodia leading out small cells with bulging nuclei. The compact nodule is observed to dissolve gradually into these small, branched, discrete cells which within a week have formed a reticular zone extending some distance out beyond the main body of the explant (Fig. 3). The reticular appearance of this zone is produced by the relatively uniform distance between the cell bodies, and by the frequent overlapping of their filamentous but branched processes. These processes do not actually unite, but are extended through the clot at different levels, so that no syncytial structure is formed. Because of these extensions in three dimensions, photography is rendered difficult. The cells under discussion bear no relationship to macrophages or monocytes, from which they can readily be distinguished by their morphology and staining reactions (Figs. 3 and 6). They are entirely unaffected by neutral red and lithium carmine, which are taken up readily by the macrophage and the monocyte. Because of their position with respect to the blood vessels of the tumor, and for other reasons which will be set forth later on, the cells emigrating from these "epithelioid" nodules will henceforth be referred to as "pericytes."

3. Mingled with the pericytes are a few fibroblast-like cells, and at the periphery of the pericyte zone are varying numbers of broad, flattened fibrocytes with numerous fine processes. Their origin is obscure; possibly they may stem from the macrophages, or possibly from the stroma of the tumor (Fig. 5-F).

4. In 10 or 12 days, capillary buds begin to push through from the explant into the zone of migrating pericytes. They advance at first as solid spikes, but later show themselves to be hollow tubes behind the foremost cells, with lumina sometimes containing blood cells (Figs. 2 and 5).

5. From the 12th day, when they appeared, to the 30th day (when cultivation was terminated), the capillary buds progressively became encrusted with pericytes. These begin to attach themselves to the capillaries as soon as the latter appear, and at some points pile up two deep. They are readily distinguishable from the endothelial cells by their smaller size and branching shape, and especially by their globular nucleus with its one or two nucleoli and richer chromatin network, as compared to the oval, paler nucleus of the endothelial cell with its larger complement of nucleoli (Figs. 2 and 5). The pericytes are indifferent to macrophages and to cotton fibers or other fortuitous inclusions in the medium, and probably also to fibroblasts in their flattened or fan-shaped form. The behavior of these cells *in vitro,* as well as their position encircling blood vessels in the sections, indicate that they are of the nature of vascular satellites. The ramose processes which characterize the pericytes are sometimes exceedingly delicate, so that they are hardly made visible by ordinary stains. But if an intensive staining method, such as silver impregnation, is used, much is brought to light that may not otherwise be seen. Compare Figure 7 made after prolonged treatment by the Bodian method for axis cylinders, with Figure 6 from a preparation stained with Mallory's phosphotungstic acid and hematoxylin. The fields are comparable and the magnifications equal. Nuclear inclusions in the form of clear, non-staining vacuoles are common among these cells, in both sections and cultures.

In addition to the infiltrative glomus tumor described above, we have cultivated two benign glomus tumors. In the latter there was not as great an amount of migration from the epithelioid areas as in the former, and the cultures did not produce encrusted capillaries. But in both, the characteristic cell composing the epithelioid trabeculae was shown to be the pericyte, discrete and branching (Fig. 8). We were not using silver impregnation methods on this tissue at the time of cultivating these benign glomus tumors, and so did not demonstrate any of the fine ramifications.

Masson, discussing the epithelioid areas in his descriptions of the normal and pathological glomus, stated that these cells form a syncytial network, interspersed by a framework of reticulin and elastin, and that they contain varying numbers of myofibrils, which sometimes extend from one cell to its neighbor, by way of their protoplasmic anastomoses.

Myofibrils are entirely absent from some of the glomus cells. According to Bailey (1935), there are no myofibrils in the glomus cells and only occasionally are groups of two or three nuclei seen without demonstrable cell boundaries.

In our cultures no reticulin was formed around the pericytes, though this was present in the sections. No myofibrils were observed in either sections or cultures of these tumors. In the cultures a few binucleate cells were seen, but, as has been stated previously, the cells were otherwise discrete and tended to range themselves at a distance from one another wherever there was room, except when in contact with a capillary, or with another pericyte upon a capillary. There seems to be a possibility that the processes of one cell passing over or wrapping around those of its neighbor in a compressed tumor nodule might produce the appearance of a syncytium such as Masson described.

Discussion

Identification of the "Epithelioid" Cell of the Glomus Tumor as the Capillary Pericyte of Zimmermann

Students of the *normal* glomus agree that the characteristic cell enveloping this short-circuiting vessel between artery and vein passes through a regular series of transitional forms, from the typical spindle-shaped smooth muscle cell of the artery to the epithelioid cell, and on across the bridge to the smooth muscle cell of the vein. In the epithelioid cells, myofibrils are reputed to be sparse or absent, though they are numerous in the coverings of artery and vein. Nevertheless, because of the function which these cells perform in occluding the glomus vessel, as well as because of the gradations observed, they are generally regarded as modified smooth muscle cells.

The characteristic cell of the glomus *tumor* appears to be the epithelioid cell at the peak of its divergence from the smooth muscle of vessel walls. Though Masson has repeatedly reported the presence of myofibrils in the pathological glomus, the majority of other observers have not. Nevertheless a contractile function seems to be indicated for these cells in the pathological as well as in the normal glomus.

The spatial and morphological transition in the glomus, from smooth muscle to epithelioid cell and back again, finds a significant parallel in the investiture of capillaries in various organs, as described by Zimmermann in 1923. This comprehensive paper (Der feinere Bau der Blutcapillaren), dealing with all the classes of vertebrates as well as with man, sets forth the thesis that "in vertebrates in general the blood capillaries are wrapped around by a special kind of cell which in both directions—towards the arteries and towards the veins—passes over

gradually, through transitional forms, into smooth muscle fibers." To this cell, together with its transitional forms, he gives the name of "pericyte." Though they do not contain myofibrils, he regards them as contractile cells, which exercise a mild regulatory function upon the blood flow through the capillaries of various organs. In man, pericytes have been demonstrated in the kidney, the liver, the heart (where they are especially numerous), the lung, the gut, the tongue and the pia mater. They were not found in the spleen. Zimmermann did not investigate the skin or the extremities.

The pericytes of Zimmermann have a compact, usually rounded nucleus surrounded by a variable amount of cytoplasm from which extend exceedingly long, branching runners which embrace the capillary and sometimes extend from one capillary to another (Fig. 5B). These processes are so thin that they can be demonstrated only by some intensive and selective staining method; for this purpose Zimmermann employed variants of the Kopsch modification of Golgi's silver impregnation technic for nerve cells. The branching processes of the pericyte grasp the capillary wall tightly and adhere so closely that no solution can be forced between. According to Zimmermann, only the secondary branches contract and not the main runner which extends lengthwise along the vessel.

These pericytes are analogous in position to the epithelioid cells of the glomus, and in function also, if Zimmermann's conclusions are correct. The apparent difference in form between pericyte and glomus epithelioid cell obscured this relationship until cultivation *in vitro* brought to light the true form of the epithelioid cell. When our cultures were treated by a modification of Bodian's silver method and compared with Zimmermann's figures of pericytes treated by the Golgi method, there emerged a strong presumption that the two kinds of cell were identical (Figs. 7 and 4). Zimmermann's investigations of pericytes extended over some 40 years and were recorded in hundreds of figures. When these are compared with the epithelioid cells which our tissue cultures make available for study, there appears little doubt that we are dealing with the same type of cell, displaying its very delicate ramifications where the population is sparse, as on the capillaries, and *in vitro,* but having them obscured where there is crowding, as in the normal and pathological glomus nodules.

Our material throws no light on the function of the epithelioid cell or pericyte. These cells were never observed to contract spontaneously *in vitro,* as we have seen striated muscle and occasionally smooth muscle contract. They were not stimulated mechanically or electrically. In the early stages of their emigration from the tumor nodules they

were often unipolar or bipolar and spindle-shaped (Fig. 9), becoming ramose later. It is impossible to say whether their processes, once extended, contracted or were withdrawn over a long period of time but there was no sudden change in form. There was no evidence of secretion, or of their functioning as *Quellzellen* (Schumacher, 1939). It should be borne in mind, however, that we have been dealing entirely with neoplastic cells, not with normal cells, and that they had no nervous connections.

The question naturally arises whether the pericyte is identical with the adventitial cell of mammalian capillaries, whose function and relationships have been the subject of much recent investigation. Zimmermann stated emphatically that the pericytes have nothing to do with the adventitial cells of the veins and arteries, which are fixed stellate connective tissue cells. They are distinct from the Kupffer cells of the bile capillaries. But since Zimmermann did not investigate the skin of the extremities, it is not possible to compare his data directly with the observations of Clark and Clark (1940) upon the blood vessels of the rabbit's ear.

From their observation of the living "extra-endothelial cells" of blood vessels by means of a transparent chamber in the ear, Clark and Clark concluded that the adventitial cells of the capillaries in this location develop from connective tissue cells in the vicinity. The longitudinally arranged adventitial cells on capillaries and small venules show no evidence of contractility. But as a capillary is transformed into an arteriole, these cells multiply and re-orient themselves transversely, becoming smooth muscle cells and contracting if they are reached by a regenerating vasomotor nerve.

In spite of the relationship of these adventitial cells to smooth muscle, there is little in Clark and Clark's figures or description to indicate that they were dealing with pericytes. In their living, unstained material they should have been able to see numerous branched processes as we see them on the pericytes in living cultures, if such processes were present on the adventitial cells. Indeed, while maintaining the general thesis that the adventitial cells of both amphibian and mammalian capillaries are inert and that the blood flow at the periphery is regulated by the smooth muscle cells of the larger vessels, Clark and Clark agreed that their evidence should not be applied to other forms and to other regions. In this connection they cited the work of Rouget (1873), Vimtrup (1922, 1923), and others on the nictitating and hyaloid membranes of amphibians, in which it appears that certain pronged adventitial cells (the Rouget cells) do exercise a contractile function and represent a primitive form of smooth muscle cell.

Two studies of normal blood vessels in tissue culture have come to our attention which suggest that the writers might have been dealing with pericytes; though this is not altogether clear, since they did not employ silver-impregnation methods. Herzog and Schopper (1931), cultivating the pia mater of guinea pigs, provided several suggestive figures; and Scriba (1935), in a study of circulatory growth in the 9-day chicken embryo, stated that adventitial cells appear in two forms: sometimes as large mesenchyme cells of fibroblastic type, and sometimes in a form resembling macrophages. A further study of such vascular satellites might prove profitable.

It seems likely that in the epithelioid cell of the glomus and in the pericyte which Zimmermann has demonstrated upon the capillaries of certain organs (and Schumacher has described on certain thyroid vessels and elsewhere), we are dealing with a type of cell which, though related to the common adventitial cell as investigated by Clark and Clark and others, is, in its differentiated form, distinct. It may possess contractility to a greater or lesser degree; but its form alone entitles it to separate consideration.

The identification of the so-called epithelioid cell of the glomus tumor as a pericyte has led us to review some of the other vascular tumors with cells arranged peripherally about the vessel wall, which are at our disposal. There are a number which on morphological grounds cannot be classified as glomus tumors and which on the other hand do not correspond with any of the accepted classes of vascular tumors. We believe that their cells may be classified either as pericytes or as adventitial cells and we propose to discuss these tumors in a future communication.

Summary

Evidence has been adduced which provides information in answer to the three queries propounded at the beginning of this paper. Glomus tumors can form not only in the cutaneous-subcutaneous zone of those parts of the body where no normal glomuses have been identified but also in deeper tissues such as joint capsule and striated muscle. A glomus tumor which displayed progressive infiltrative growth has been described indicating that not all of these tumors are localized and encapsulated. However, we do not believe that sufficient evidence exists to establish the fact of metastasis. The "epithelioid" cell of the glomus tumor has been identified as the pericyte of Zimmermann. Since this cell has been demonstrated in many parts of the body, this identification offers a satisfactory explanation for the occurrence of glomus

tumors in those regions of the body where normal glomuses have never been found.

NOTE: We wish to acknowledge our indebtedness to Miss Irene Bokoff for her technical assistance in the preparation of the tissue cultures.

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DESCRIPTION OF PLATES

PLATE 26

FIG. 1. Photomicrographs of infiltrating glomus tumor.

A. The peripheral zone with sprouted capillaries. Some capillaries are simple endothelial-lined tubes with a collagenous sheath; others have "epithelioid" cells (pericytes) adherent to the sheath.

B. Endothelial-lined tumor vessels surrounded by several layers of "epithelioid" cells (pericytes) with sharp cell margins and clear zones around their nuclei. There are reticulin fibers between the pericytes.

C. A circumscribed tumor nodule of collapsed endothelial tubes surrounded by massed "epithelioid" cells (pericytes). One of them shows a nuclear vacuole.

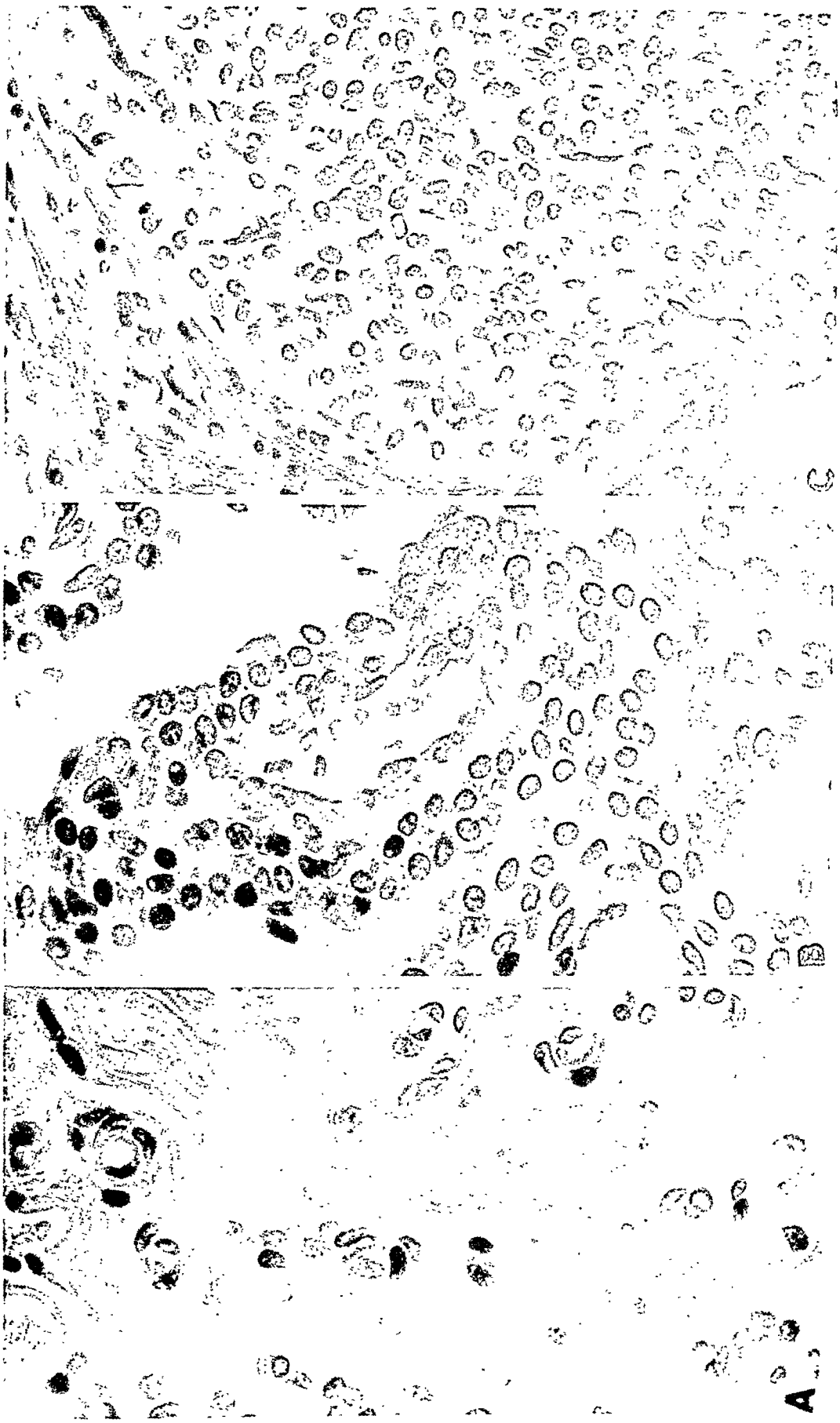


PLATE 27

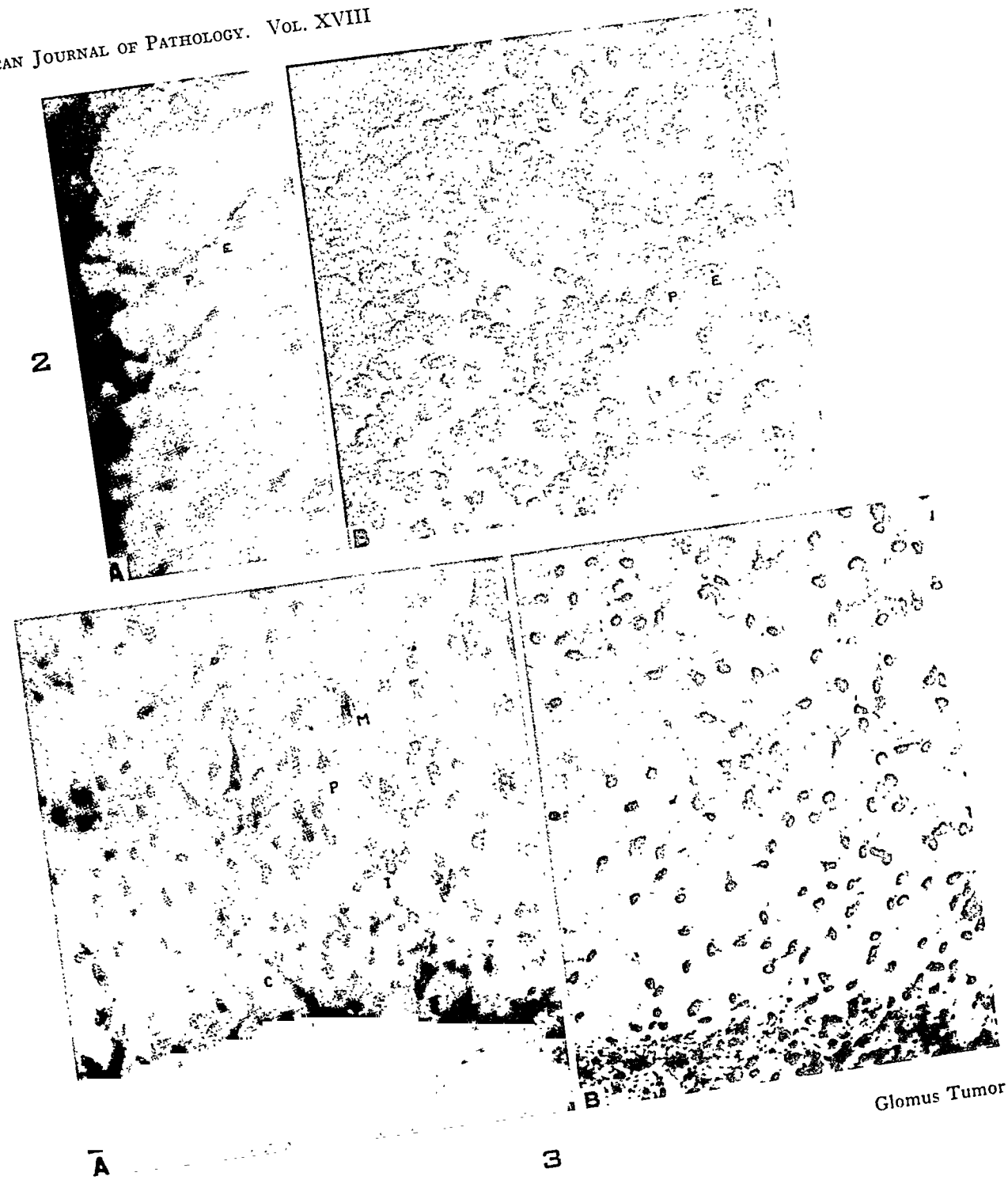
FIG. 2. A. Tissue culture from "epithelioid" nodule. Twenty days *in vitro;* Zenker's fixation, phosphotungstic acid hematoxylin stain.

B. Similar material. Twenty-eight days *in vitro.* Kopsch fixative, phosphotungstic acid hematoxylin stain. E, endothelial nucleus; P, pericyte nucleus.

FIG. 3. A. Tissue culture from tumor nodule composed mainly of "epithelioid" cells. Ten days *in vitro;* Zenker's fixation, phosphotungstic acid hematoxylin stain. C, capillary bud; I, nuclear inclusion body; M, macrophage; P, pericyte or "epithelioid" cell.

B. Similar material, 24 days *in vitro,* 4 per cent formaldehyde solution, Bodian silver impregnation.

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PLATE 28

FIG. 4. A. Redrawn from Zimmermann (1923). Capillary pericyte with secondary processes contracted; from heart of 43-year-old man.

B. Pericytes from "epithelioid" nodule of infiltrating glomus tumor (18780). Twenty-four days *in vitro,* Bodian silver impregnation.

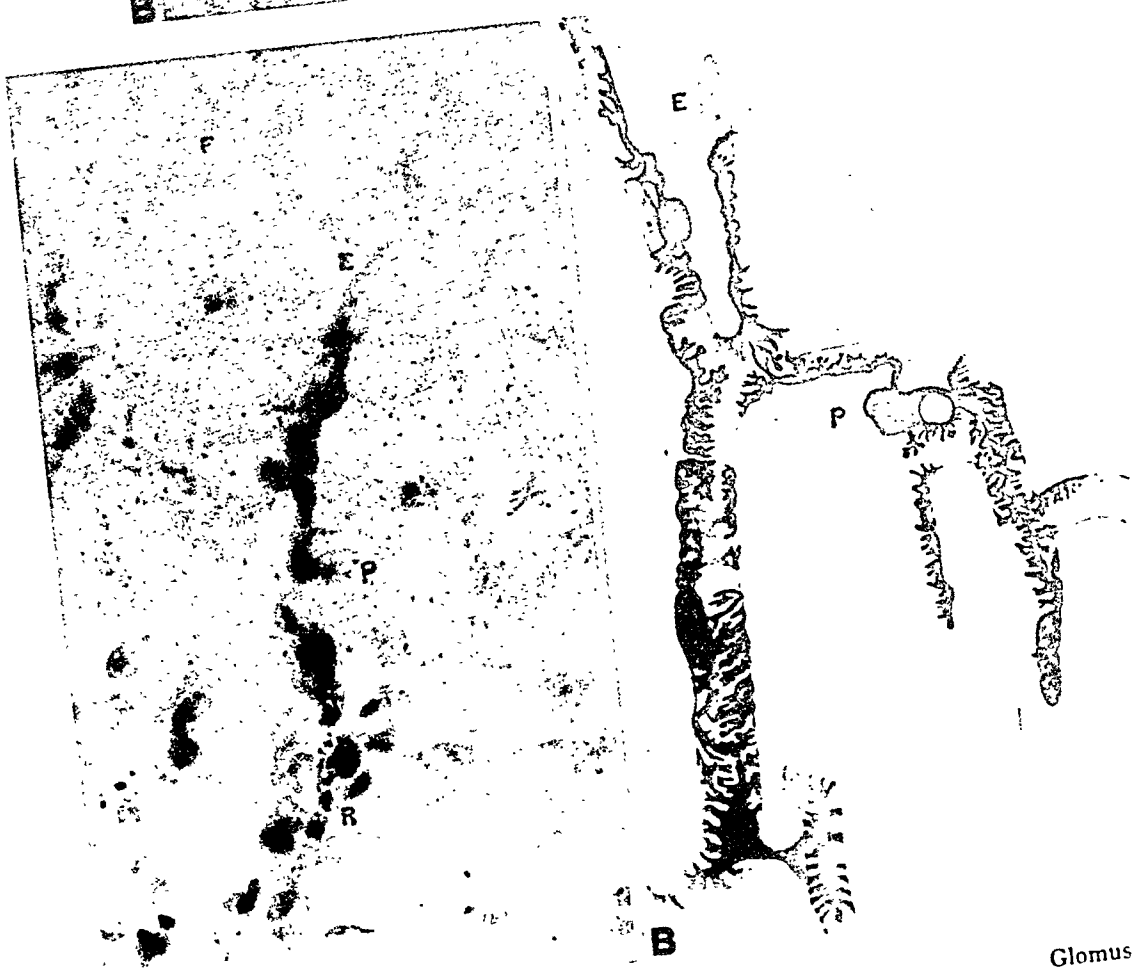
FIG. 5. A. Tissue culture from "epithelioid" tumor nodule. Twenty-eight days *in vitro;* Kopsch fixative, toluidine blue stain. E, endothelial cell; F, fibrocyte; P, pericyte; R, red blood cell.

B. Redrawn from Zimmermann (1923). Capillary and precapillary pericytes, in the heart of a 43-year-old man.

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A

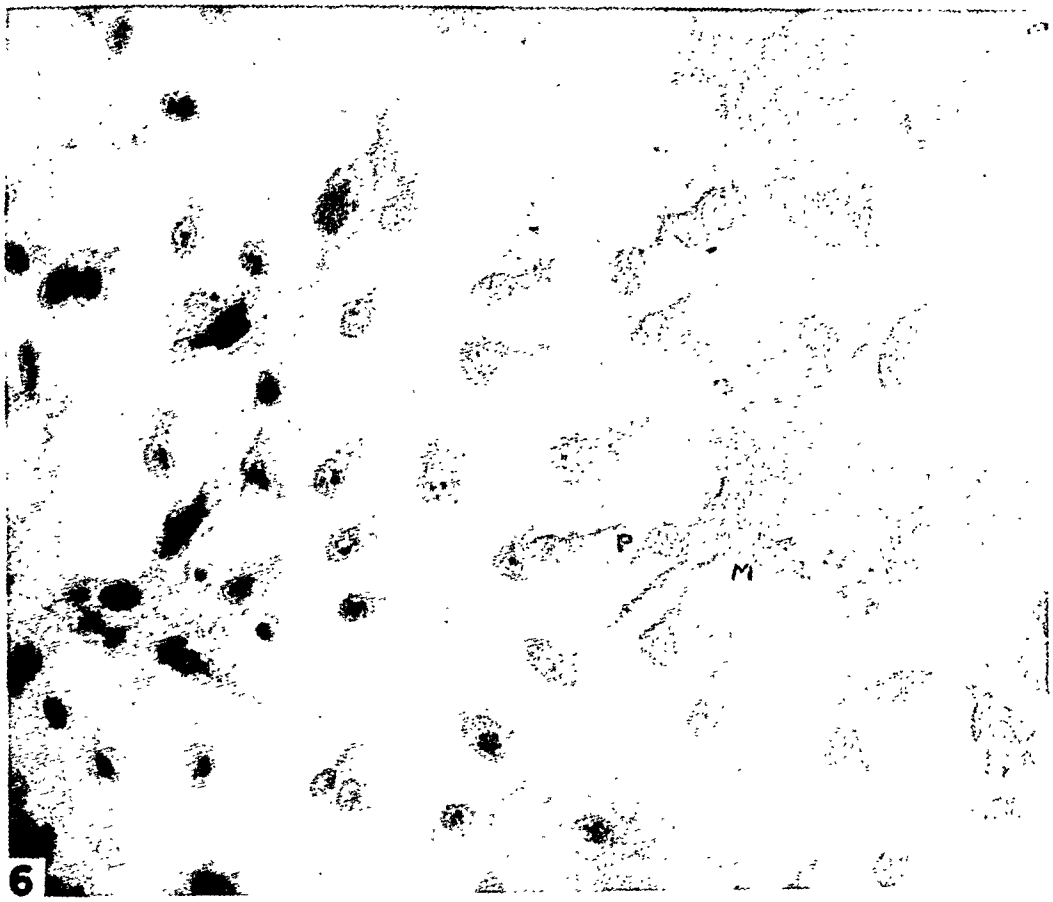
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Glomus Tumor

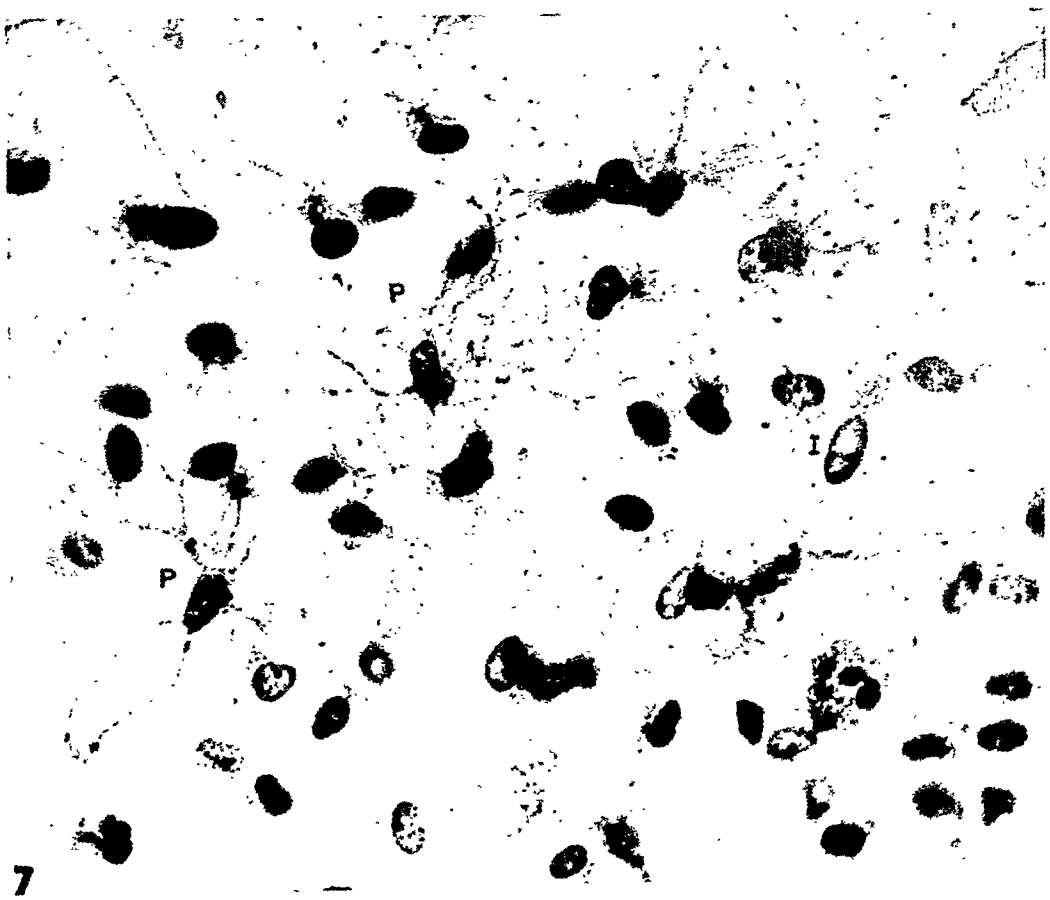
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PLATE 29

FIG. 6. Same culture as in Figure 3A; higher magnification.
FIG. 7. Same culture as in Figure 3B; higher magnification.



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Glomus Tumor

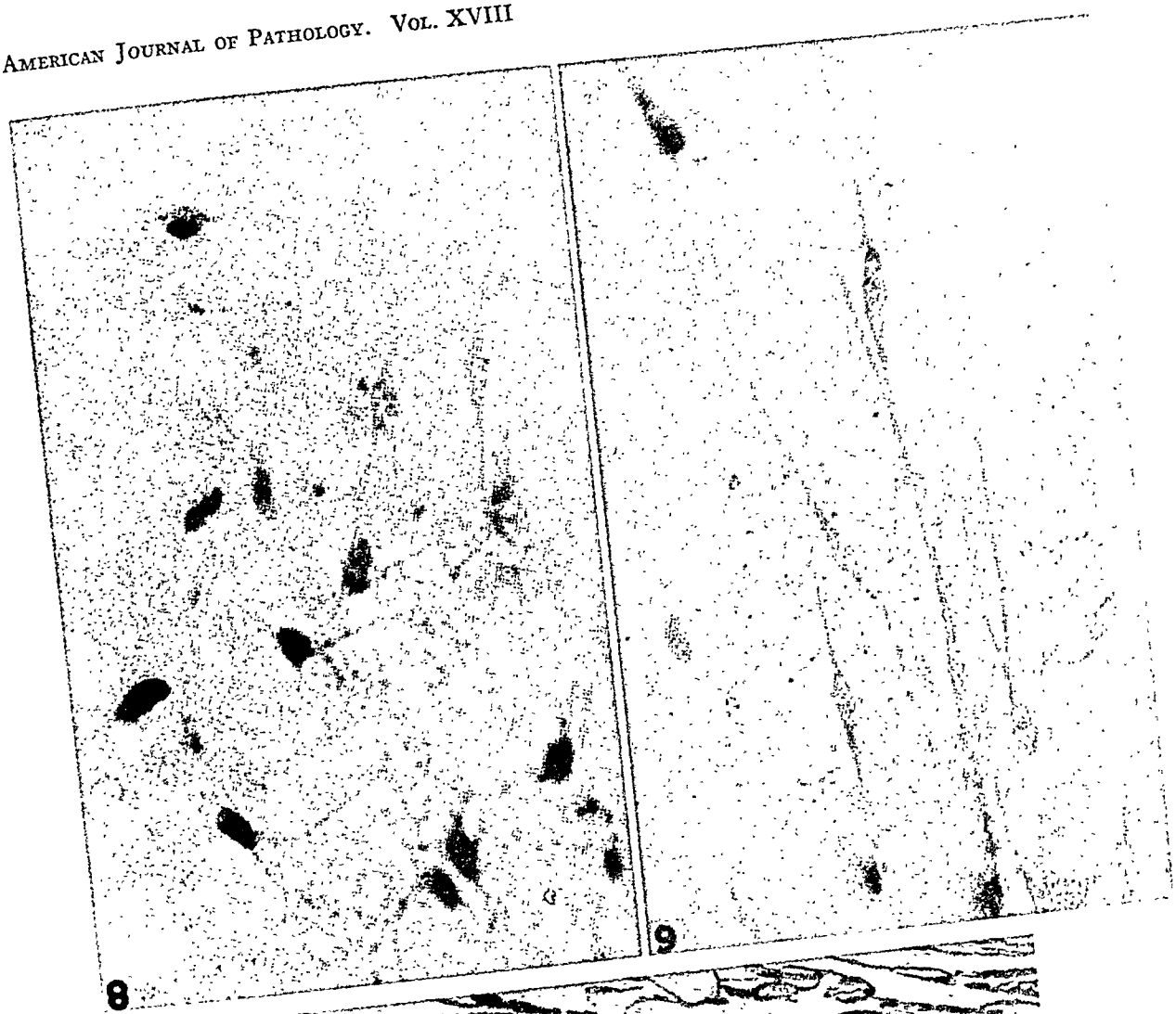
PLATE 30

FIG. 8. Tissue culture from a benign, circumscribed glomus tumor from nail-bed of finger (72078). Six days *in vitro;* Zenker's fixation, phosphotungstic acid hematoxylin stain.

FIG. 9. Tissue culture from infiltrating glomus tumor (18780), "epithelioid" nodule; showing pericytes in monopolar and bipolar spindle-shaped form. Five days *in vitro;* Zenker's fixation, phosphotungstic acid hematoxylin stain.

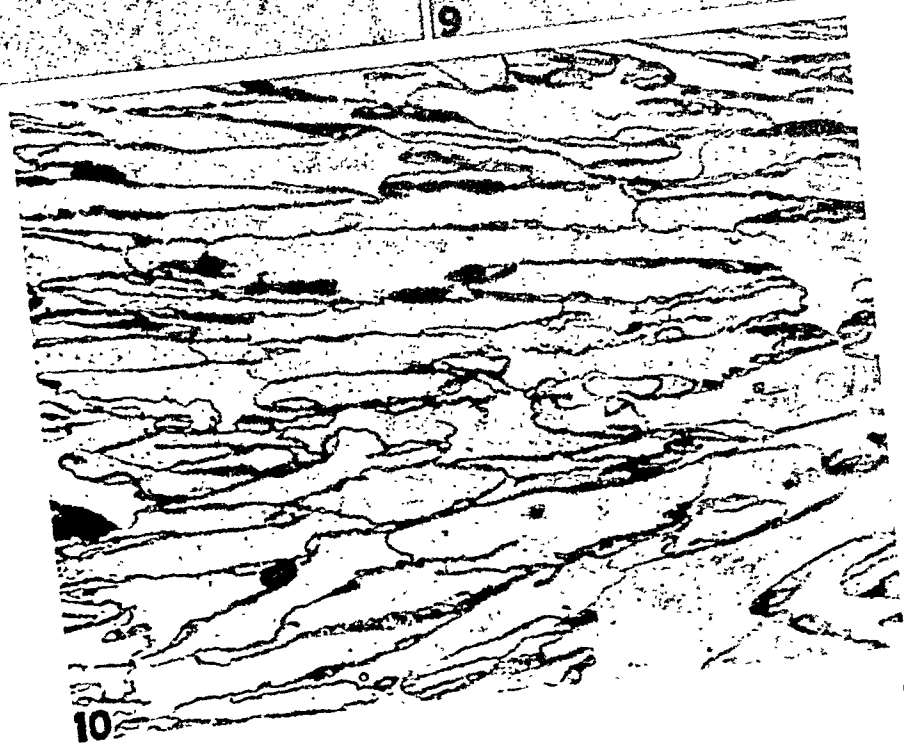
FIG. 10. Tissue culture from external zone of infiltrating glomus tumor (18780). Twenty days *in vitro;* silver nitrate, toluidine blue.

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Glomus Tumor

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NON-OSTEOGENIC FIBROMA OF BONE *

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Not infrequently, one encounters in bone a lesion which we conceive as a benign marrow-connective-tissue tumor and which, because the basic tissue does not undergo osseous metaplasia in the course of the lesion's development, we are calling "non-osteogenic fibroma of bone." Its usual site is the shaft of some long bone (generally one of a lower limb) not far from an epiphyseal cartilage plate, and the lesion does not necessarily traverse the entire diameter of the bone in the affected area. Grossly, the lesion appears as a single focus or a group of smaller adjacent foci of yellowish or brownish fibrous tissue. Histologically, its essential pattern is composed of whorled bundles of spindle-shaped, connective-tissue cells loosely interspersed with small multinuclear giant cells, but in many places within a lesion collections of foam cells may be seen, along with other variations in histologic detail.

The lesion in question has previously been interpreted in a good many different ways; for instance, as "giant-cell variant of bone cyst or osteitis fibrosa," "healing variant of giant-cell tumor," "xanthic variant of giant-cell tumor," "solitary xanthoma or xanthogranuloma of bone" (usually implying a limited expression of Hand-Schüller-Christian's disease), and "fibrous osteomyelitis." It is the purpose of this presentation (founded on the observation of ten cases) to define and interpret "non-osteogenic fibroma of bone" as a clear-cut entity deserving recognition as such on the basis not only of its pathology but also of its clinical and roentgenographic manifestations. Although the term "fibroma of bone" does appear here and there in the literature, the case reports under that heading which we have studied have consistently been found to relate to a lesion having, as basic elements, both osseous and connective tissue, instead of to a connective-tissue lesion specifically *not* containing osseous elements. These fibro-osseous lesions most often represent the so-called "ossifying fibroma" or "fibrous osteoma" of bone, though some of them apparently represent solitary expressions of a dysplastic connective-tissue lesion of bone which we would call "fibrous dysplasia"—a lesion likewise not corresponding to "non-osteogenic fibroma."

* Received for publication, June 25, 1941.

Clinical Aspects

Age and Sex Incidence and Localization

In respect to age, our ten subjects ranged between 6 and 21 years, and all but two were between 8 and 16 years. As to sex, there was an even distribution of the cases. In regard to localization, in all ten the lesion was in a long tubular bone. Specifically, it was in a tibia in four patients, a fibula in four, a femur in one and an ulna in one, thus involving a long bone of a lower limb in all but one case. Within the particular bone affected, the lesion was always in the shaft, and almost always limited to the upper or lower third. However, there was always an inch or two, though seldom much more, of unaffected shaft between the lesion and the nearer epiphyseal cartilage plate (Figs. 1 to 6).

It is difficult to make a direct comparison, in respect to incidence and localization, between the findings in our cases of non-osteogenic fibroma of bone and the findings in apparently similar cases from the literature as reported under other names. This is so because the data supplied often do not permit one to sort out confidently all the pertinent cases from among others that are usually described along with them. Nevertheless, even against the obscure background of the general literature and the confusion of classification, the predilection of the lesion in the apparently relevant cases for older children and adolescents and for the shafts of long bones near but not at the epiphyses stands out. Though our records do not include any instances of non-osteogenic fibroma of bone in other than long tubular bones, there is reason to suspect that the lesion does occasionally appear also in other bones.

Clinical Findings

In most of our cases, the complaints were of only a few weeks' or months' duration before admission to the hospital, although this does not mean that the lesion itself was not of longer standing. It is probable that the lesion is one which progresses very inconspicuously and may lie dormant for some time before attention is drawn to it. About half of the patients reported their difficulty as beginning with some trauma of moderate severity to the general region in which the bone lesion was subsequently discovered. Thus, after a sprain, kick, or fall these patients suffered from pain and swelling of an ankle, knee, or wrist. In these cases, palpation revealed a point of bone tenderness and sometimes even of bone swelling, and when a roentgenogram was made the lesion was discovered. Most of the other patients, while giving

no history of trauma, likewise had pain and swelling of a joint, not of long standing, as their chief complaint. In these cases, too, it was only the roentgenogram that directed attention to the lesion in one of the bones entering into the formation of the joint which was the focus of complaint. In one of the cases in which the lesion was in the tibia, this lesion was discovered incidentally to roentgenographic examination of the femur for an osteogenic sarcoma. Altogether, there is nothing distinctive or characteristic about the clinical findings in cases of non-osteogenic fibroma of bone, so that one is dependent upon the roentgenogram for even a presumptive diagnosis.

Roentgenographic Findings

The usual location of the lesion in the upper or lower third of a long bone shaft at some distance from the nearer epiphyseal cartilage plate has already been emphasized. In this connection, it should also be noted that often the lesion does not extend across the entire diameter of the affected shaft area. It failed to do this in the four cases in which it was in a tibia and the one in which it was in a femur. It did do so in the four cases in which it was in a fibula and the one in which it was in an ulna. Thus, whether it does or does not extend all the way across the diameter of the bone depends upon the width of the shaft in the affected area, the lesion tending to be rather small and consistently being longer than it is wide. When it extended all the way across the shaft, the latter was found bulged out, uniformly or at least on one side. Even without reaching all the way across, it also occasionally bulged out the shaft in one place. Furthermore, when this was not the case, the lesion was still eccentric, that is, it hugged the cortex on one side (Figs. 1 to 6).

In the fibula and ulna (that is, in the cases in which the fibroma extended across the width of the shaft and expanded it somewhat) the lesion was usually about 3.8 cm. in length. It appeared as a more or less subdivided area of rarefaction, showing confluent or distinct locules. Some of the thin dividing "partitions" which it presented cast rather dense shadows. The cortex delimiting the area as a whole tended to be thinned and expanded over much of its scope, but still cast a clear-cut shadow. In a case showing a transverse infraction line, the cortex on the side of the infraction was found thickened by new bone apposition. Like the lesions extending across the width of the shaft, the eccentric lesions (those in the tibia and femur) were usually also not more than 3.8 cm. in length and less than this in width. When they did not expand the cortex upon which they abutted, this cortex was sometimes found sclerosed. Toward the medullary side, the lesion

was outlined by an encapsulating shell, usually rather thin but casting a dense shadow, and, as a rule, one or more "partitioning" shadows traversed the lesion irregularly.

Even from the roentgenogram alone one can often make the correct diagnosis. Certainly a small, eccentric, loculated lesion found abutting on the cortex of the shaft of a long bone, outlined by an encapsulating shell of bone on the medullary side, and not associated with notable thickening of the cortex, is most probably a non-osteogenic fibroma. So also (though a little less surely) is a small lesion located in, and expanding, the shaft of a fibula or ulna and appearing as a loculated area of rarefaction, although it should be borne in mind that such an area occasionally represents a solitary unicameral bone cyst. However, in any event, the definitive diagnosis must rest upon pathologic examination of the tissue occupying the affected shaft region.

Treatment

Non-osteogenic fibroma of bone is readily amenable to treatment. The cases discussed in this paper were all treated surgically, the procedure in most cases being thorough curettement of the lesion. In three cases in which the lesion was in the fibula, subperiosteal resection of the affected part of the bone was done. This seemed the easiest way of completely eradicating the focus of the disease in these particular cases, though, even in slender long bones, resection may not always be necessary. There were no recurrences. None of the patients received postoperative radiation therapy. Whether the lesion would be amenable to radiation therapy alone (that is, without surgical intervention) we cannot say. Of course, without the histologic examination of tissue made possible by such intervention it would be difficult to know whether the lesion being so treated actually represented a non-osteogenic fibroma of bone.

Pathology

The periosteum of the affected portion of the shaft is not particularly thickened, except at the site of an infraction undergoing repair. On exposure of the medullary activity, the lesion, as already noted, is likely to be found to be eccentric and abutting upon the cortex on one side only if the long bone affected is a thick one, or, on the other hand, extending across the entire diameter of the shaft if it is a slender one. The lesion usually consists of several more or less discrete but adjacent foci of tough tissue having a fibrous character (Figs. 7 and 8). The color of this tissue is brownish or yellowish, and, though some lesions may be more or less uniform in color throughout, others present a more mottled appearance, created by a mixture of yellowish and

brownish foci. As to the cortex of the shaft neighboring upon the brown-yellow tissue of the lesion, this may be found eroded and thinned in some places and abnormally thickened in others. Furthermore, each focus may be outlined in part by a thin shell of sclerotic bone, and some of the individual foci may also be separated from each other by bits of sclerotic spongiosa.

On microscopic examination, it appears that the general pattern of the stroma of the lesion consists of whorled bundles of connective-tissue cells (Figs. 9 and 10). However, the cellularity of the stroma varies from one lesion to another or from one focus to another within the same lesion. In accordance with the relative gross brownness or yellowness of the tissue, there is also variation in regard to the lesion's vascularity, although, on the whole, the latter is not great.

Thus, in a distinctly brownish lesion or focus, the stromal connective-tissue cells are spindle-shaped and closely compacted, being interspersed with but little collagenous intercellular material. Many of the stromal cells are likely to contain granules of hemosiderin in their cytoplasm, and it is mainly this that accounts for the brownish color of the lesion or focus as a whole, although some scattered capillary hemorrhages may also contribute to it. Irregularly dispersed among the stromal cells are small, often elongated multinuclear giant cells. These cells, sparse on the whole, may be more numerous and clustered together in some fields and especially about areas of recent capillary hemorrhage. The giant cells seem to be formed through fusion of the spindle-shaped stromal cells, and, like the latter, many of them contain granules of hemosiderin in their cytoplasm (Fig. 11).

In a distinctly yellowish lesion or focus, large and small nests of lipoid-containing foam cells are seen, admixed with and encircled by the stromal tissue (Fig. 12). The latter then consists of rather collagenous, spindle-shaped connective-tissue cells in winding thick strands or whorled bundles, honeycombed by the lipoid cells. It can be shown that the lipoid cells arise through conversion of the spindle cells into lipophages, and that the lipoids contained within the latter are, to a large extent, of the nature of cholesterol esters. On the whole, the more yellow the lesion or focus, the more lipophages does it contain and the more collagenous does the intervening stromal tissue appear; and, furthermore, the less does it show of hemosiderin pigment in the stromal cells, or of multinuclear giant cells among them. Why the disappearance of the hemosiderin pigment and giant cells should parallel the appearance of foam cells in the lesion we do not know, but the fact that it does so is clear from the findings in areas representing intermediary stages of yellowness or brownness.

Thus, in an individual lesion, one may see fields in which the stromal

connective-tissue cells are rich in hemosiderin and interspersed with giant cells, and other fields in which pigment-bearing cells and giant cells are sparse or absent and foam cells are numerous. However, in about half of our cases, the entire lesion failed to show any lipoid at all, although the latter was sought for in frozen sections of material stained for fat, and foam cells were looked for in paraffin sections prepared from many areas of each lesion. Hence, as will be shown more fully later on, it seems clearly unjustifiable to lay emphasis upon the inconstant lipoid element by calling the condition a xanthoma or xanthofibroma of bone.

Furthermore, none of the lesions, of course, showed evidence of osteogenesis as a feature of the cytology, and indeed the lack of bone formation within these lesions is a consistent and striking finding. It is true that individual foci may be walled off or delimited at their periphery by a narrow zone of bone. Also, abutting upon the cortex of the shaft, the lesion may even provoke the former to thicken in some places, just as, in other places, it may erode it. However, in either case, such bone formation represents a response of the neighboring tissue to the lesion, and is not a feature of the lesion itself.

Discussion

It is realized that calling the lesion under consideration "non-osteogenic fibroma of bone" raises questions of classification and nomenclature. As a matter of fact, the Surgeon General's Catalogue and the Index Medicus list hardly any references to "fibroma of bone" and none to "non-osteogenic fibroma of bone." Furthermore, the various textbook classifications of bone tumors include no such category as "non-osteogenic fibroma." References to "ossifying fibroma," "osteofibroma," or "fibrous osteoma" are considerably more common, but these terms, indicating as they do that one of the inherent elements in the lesions so classified is osseous tissue, distinguish these lesions from the one we are considering here. How, then, is that lesion, which is by no means a rare one, recorded in the literature? Most commonly, cases representing non-osteogenic fibroma of bone are found described as instances or variant forms of so-called localized osteitis fibrosa and as instances or variant forms of giant cell tumor of bone.

There can be no doubt that some, though by no means all, of the lesions which Geschickter and Copeland,[1] for instance, discuss as "giant cell variants of the bone cyst in the metaphyseal ends of the long bones" (p. 268) represent what we are calling "non-osteogenic fibroma of bone." Also, their illustration (p. 300, Fig. 195) of the histology of what they call "the giant cell variant of osteitis fibrosa" would do per-

fectly as an illustration of the lesion we are describing. Altogether, their terminology implies that the lesion in question is related both to solitary bone cyst and to giant cell tumor of bone, and that it actually represents something intermediary between them (p. 269). To follow their reasoning at all, one must bear in mind the opinion of these authors that solitary bone cyst (or osteitis fibrosa) and giant cell tumor are closely related lesions which have a common basis in an abnormal hyperplasia of osteoclasts at sites of endochondral ossification (pp. 289 and 308).

The reasoning of Geschickter and Copeland [1] which has just been outlined contains several fallacies. One is that bone cyst (osteitis fibrosa) and giant cell tumor are pathogenetically related lesions. Indeed, our own findings [2] indicate that solitary bone cyst starts apparently on the basis of a local disorder of development and growth of bone and certainly does not represent a focus of "osteitis fibrosa" which has become cystic. Furthermore, a solitary bone cyst usually has few if any tissue masses adherent to its wall, and such as may be present show clearly that they have their basis in the organization of hemorrhage. Again, our own findings in regard to giant cell tumor of bone [3] indicate that the basic cell of that lesion is not the giant cell but the stromal cell, and that this stromal cell is, of course, not an osteoclast but rather an immature marrow-connective-tissue cell. Finally, though in non-osteogenic fibroma the basic histologic pattern is that of intermingled stromal cells and giant cells, its resemblance to the giant cell tumor is only superficial. In the former as contrasted with the latter, the stromal cells are small and very spindly and show a strong tendency to collagenization and lipoid impregnation; the giant cells are small and sparse; and the lesion as a whole often provokes a perifocal osteosclerosis.

These very characteristics of the cytology of non-osteogenic fibroma have led other observers to regard this lesion as representing either a healing form [4] or a xanthic variant [5] of giant cell tumor. This idea is not valid either. In particular, non-osteogenic fibroma is observed most often in subjects below 20 years of age, while genuine giant cell tumor is rarely observed in subjects below this age. Furthermore, the fibroma nearly always begins in the shaft of a long bone, not far from an epiphyseal plate, but does not tend to extend into the epiphysis, while giant cell tumor usually begins in an epiphysis of a long bone though it tends to extend to the shaft. Again, non-osteogenic fibroma is usually a small lesion in comparison with a giant cell tumor. In addition, the scattered multinuclear giant cells which are often present among the stromal connective-tissue cells do not argue against designation of the lesion in question here as a fibroma, since medullary connective tissue,

even when rather mature, has a strong inherent tendency to form giant cells wherever it is proliferating. On the other hand, giant cells are likely to be absent in a non-osteogenic fibroma, or parts of it, where the lesion has undergone much lipoid transformation and collagenization, apparently in association with a diminution or regression of its growth activity. Finally, none of the giant cell tumors which we have studied showed substantial lipoid transformation or collagenization, or provoked a perilesional osteosclerosis such as would offer even slight justification for linking non-osteogenic fibroma with giant cell tumor or, specifically, for regarding the former as a healing or perhaps xanthic variant of the latter.

There can be no doubt that examples of non-osteogenic fibroma of bone have, in the past, been described as solitary xanthoma, xanthofibroma, or xanthogranuloma of bone and such have usually been conceived in this connection as solitary expressions of lipoid granulomatosis or Hand-Schüller-Christian's disease. Pertinent cases have been described by Phélip,[6] Bahls [7] and Burman and Sinberg.[8]* This interpretation is likewise invalid, in our opinion. It is tempting because, in a non-osteogenic fibroma, many of the stromal cells may become converted into lipoid-containing foam cells. However, it should be borne in mind that in about half of our cases the entire fibroma failed to show any foam cells at all, although a careful and systematic search for them was made. Furthermore, even in those few lesions in which foam cells were abundant in some areas they were still absent in others, and in any case their presence was not associated with an inflammatory reaction. Altogether, when lipoid-containing fibromas of bone are compared histologically with genuine lesions of Hand-Schüller-Christian's disease, it becomes clear that there is no proper basis for regarding them as identical lesions.

In fact, we regard as very lax the current tendency to label lesions in bone as expressions of Hand-Schüller-Christian's disease merely because they contain some foam cells. For instance, Farber,[9] who observed foam cells in the lesions of eosinophilic granuloma of bone,[10] interpreted the latter condition as a variant of Hand-Schüller-Christian's disease. Other examples are provided by Snapper [11] and Landoff,[12] who, likewise observing a few foam cells in a lesion of fibrous dysplasia,[13] labelled that condition, too, as a variant of xanthomatosis or lipoid granulomatosis. If one used this line of reasoning, he would

* The article by Burman and Sinberg relates to one of the cases included in the present discussion and quotes one of us (H. L. J.) as having made an anatomic diagnosis of "lipoid granulomatosis (xanthoma) of bone" in that case. Aside from the interpretation given to the report we originally made in this case, we wish to record that we would not make that diagnosis now.

also have to say that certain cases of suppurative osteomyelitis, for instance, represent a variant of Hand-Schüller-Christian's disease, merely because in chronic stages they may reveal some foam cells in the inflammatory granulation tissue.

On attacking the question of nomenclature and classification from the opposite angle, it appears that though the term "fibroma of bone" has sometimes been used,[14] it seems not to have been applied to the lesion in question here. The term has most often been applied to lesions (particularly in the jaw bones) of the same character as those which have also been denoted as ossifying fibroma or fibrous osteoma. It has likewise been applied (again particularly in relation to the jaw) to lesions of the nature of solitary foci of fibrous dysplasia. In relation to other skeletal regions, lesions designated as fibroma of bone nearly always represent foci of fibrous dysplasia. Thus, for instance, the lesion described by Levinthal and Kirshbaum [15] as "fibroma of the metacarpal bone" is a clear-cut example, in respect to both gross and microscopic appearance, of fibrous dysplasia of the bone in question. As they stated, the expanded and otherwise modified metacarpal bone was substantially occupied by grayish white connective tissue showing some bony metaplasia and some giant cells, occasionally collected around bony trabeculae. The lesion which Mustakallio [16] referred to as "central bone fibroma" likewise represents fibrous dysplasia. This author stated that at the site of a central fibroma of bone the interior of the bone is filled, at least to a major extent, by a mass of connective tissue which is loose and reticular in some places and compact and collagenous in others. It is precisely this composition (usually with the addition of metaplastically formed bony trabeculae) that gives distinctive character to the lesion which we have denoted as fibrous dysplasia of bone—a lesion which affects only one bone or part of a bone in some cases and several or even many bones in others.

In choosing the term "non-osteogenic fibroma of bone" for the lesion under discussion we have meant to imply, as already noted, that the lesion represents a benign tumor, that it arises from the connective tissue of the marrow, and that in the course of the tumor's development the proliferating connective tissue does not undergo osseous metaplasia. We are aware that there are many who would question the propriety of calling the lesion a tumor at all. Indeed, some would argue that it represents merely an inflammatory condition and specifically something like a "fibrous osteomyelitis." However, its conception as an osteomyelitis (even in burnt-out form) is not made plausible by the addition of the modifying term "fibrous." This is merely an evasion, for there is certainly nothing in the histology of the lesion to indicate that it has, or

has ever had, a basis in inflammation. Nor is there anything in its histology to indicate that it may have a basis in reparative processes taking place after local trauma. On the other hand, a lesion which thus presents no vestiges of inflammation or reparative response, but which shows a cytologic pattern interpretable as having developed through the apparently unprovoked proliferation of autochthonous connective tissue must, on the basis of oncological usage, be classified as a tumor. However, it is a tumor of rather limited growth capacity. The absence from it of striking cellularity, mitotic division figures and nuclear atypism militates (like the clinical course) against calling it a fibrosarcoma, even of low grade, as is probably sometimes done.

Summary

The lesion which is the subject of this paper is being called "non-osteogenic fibroma of bone" because we hold it to be a benign tumor formed from matured marrow connective tissue and not containing osseous trabeculae as an integral feature. In regard to the clinical findings, we noted that most of the subjects are older children or adolescents and pointed out the lack of characteristic clinical manifestations in connection with the disorder. We also noted that the usual site of the lesion is the shaft of a long tubular bone (most commonly of a lower limb), not far from the nearer epiphyseal cartilage plate. It was observed that the lesion tends to be a small one and may not traverse the entire diameter of the affected bone, especially if the latter is not slender. Accordingly, it was remarked that the lesion may show up roentgenographically as a sharply delimited, eccentric, somewhat loculated area of rarefaction, hugging and even bulging out the cortex on one side or, on the other hand, as a multilocular area of rarefaction traversing the bone and even bulging it out on both sides. As to its pathology, the lesion was described as consisting grossly of several discrete but contiguous yellow-brown fibrous foci whose basic microscopic pattern was found to be made up of whorled bundles of spindle-shaped connective-tissue cells loosely interspersed with small multinuclear giant cells, though, in some lesions, areas containing foam cells may also be present and even prominent. As to treatment, it was pointed out that thorough curettement or block resection of the affected area is all that is needed to abolish the disorder. Finally, we have tried to show why "non-osteogenic fibroma of bone" does not represent bone cyst (osteitis fibrosa) or giant cell tumor even in variant form, nor lipoid granulomatosis (Hand-Schüller-Christian's disease) in the form of a solitary lesion, nor a focus of "fibrous osteomyelitis."

Note: We are indebted to Drs. Henry Milch and Isaac Reitzfeld for permission to reproduce the roentgenograms shown in Figures 4 and 5 respectively.

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DESCRIPTION OF PLATES

PLATE 31

FIG. 1. Roentgenograph showing a lesion of small size, located in the tibia, about 4.5 cm. below the site where the upper epiphyseal cartilage plate had been, and hugging the cortex posteriorly. A delimiting shell of bone is seen, especially on the medullary side of the lesion. The patient was a young man, 21 years of age, in whom the lesion was asymptomatic and was discovered in connection with examination of the femur on the same side for an osteogenic sarcoma.

FIG. 2. Roentgenograph showing a lesion of about the same size and location as that shown in Figure 1. The cortex of the tibia toward the distal end of the lesion is somewhat thickened. The patient was a boy, 16 years old, who complained of pain in the knee of only 2 weeks' duration, starting after a football game.

FIG. 3. Roentgenograph showing a somewhat larger and longer lesion also abutting on the posterior wall of the cortex of a tibia. The upper limit of the lesion was 6.3 cm. below the upper plate of the tibia. The lesion appears multilocular with a delimiting shell of bone about it. The patient was a boy, 15 years of age, who went to the hospital because of pain and disability of the knee, of one month's standing, without antecedent trauma.

FIG. 4. Roentgenograph showing a lesion in the lower portion of the shaft of a fibula. The lower end of the lesion is about 2.5 cm. above the corresponding epiphyseal cartilage plate. The lesion appears multilocular with expansion of the diameter of the bone in a large part of the affected area. From the roentgenogram alone one might suspect, quite plausibly, that he was dealing with a solitary unicameral bone cyst, for instance, but actually the entire affected area was found filled by yellow-brown fibrous tissue (see Figs. 8 and 9). The patient was a girl, 10 years of age, who complained of pain of about 4 months' standing, in an ankle, and dated her difficulty from a kick in that area.

FIG. 5. Roentgenograph showing a lesion resembling that shown in Figure 4 but located in the lower portion of the shaft of an ulna. The patient was a boy, 8 years old, who complained of mild local pain, of 2 months' standing, said to have appeared first after a fall.

FIG. 6. Roentgenograph of a lesion in the upper portion of the shaft of a fibula. The upper end of the lesion is about 1.3 cm. below the corresponding epiphyseal cartilage plate. Periosteal new-bone apposition, representing a response to a transverse infraction, is seen on the surface of the cortex. (Compare with photograph of the specimen, as shown in Fig. 7). The patient was a girl, 7 years of age, whose history included pain, of about 2 years' standing, in the upper part of the left leg.

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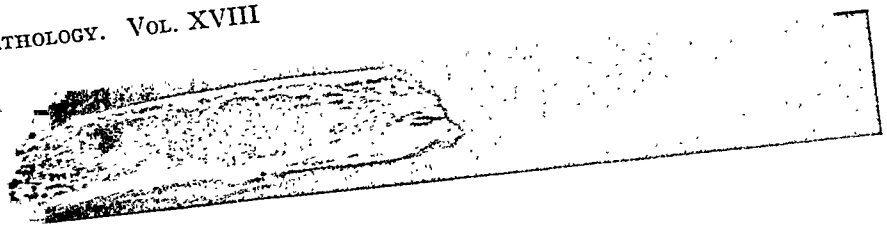
PLATE 32.

FIG. 7. Photograph of the resected portion (split longitudinally) of the affected fibula shown in Figure 6. Two adjacent foci of disease are seen which correspond precisely to the areas of rarefaction shown in the pertinent roentgenograph. For cytologic details, see Figures 10 and 11.

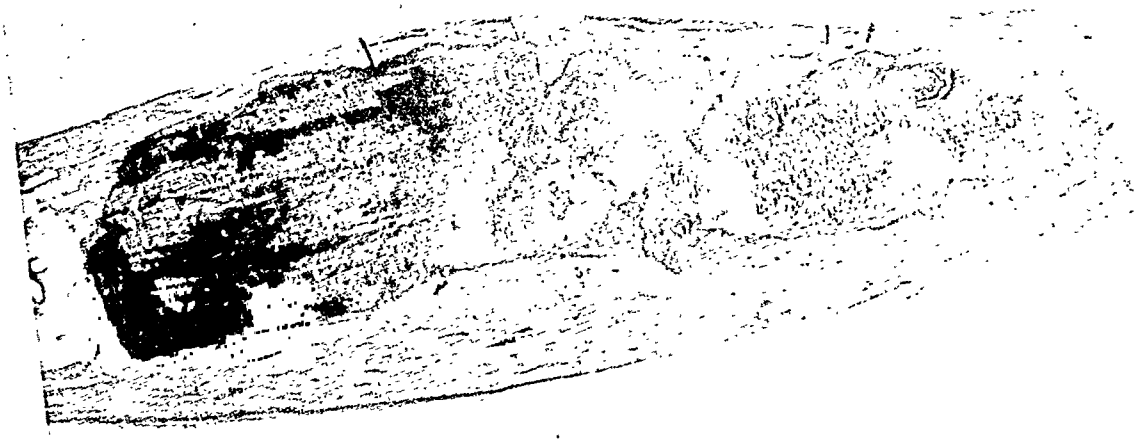
FIG. 8. Photomicrograph of a section prepared from the resected portion (split longitudinally) of the affected fibula shown in Figure 4. Although tissue is seen to fill the medullary cavity in the affected area, and the pertinent roentgenogram might suggest a cyst, there is actually no cavity in the region in question. The tissue of the non-osteogenic fibroma is very dark in the picture, not only because it was itself quite brown, but also because the section was rather thick. For some histologic details of the tissue composition in this lesion, as shown in a thinner section, see Figure 9. × 3.

FIG. 9. Photomicrograph showing the general cytologic pattern of the lesion shown in Figure 8. A whorled arrangement of the stromal cells is seen, and scattered, larger dots which are the multinuclear giant cells. There is complete absence of osseous trabeculae within the connective-tissue focus. × 8.

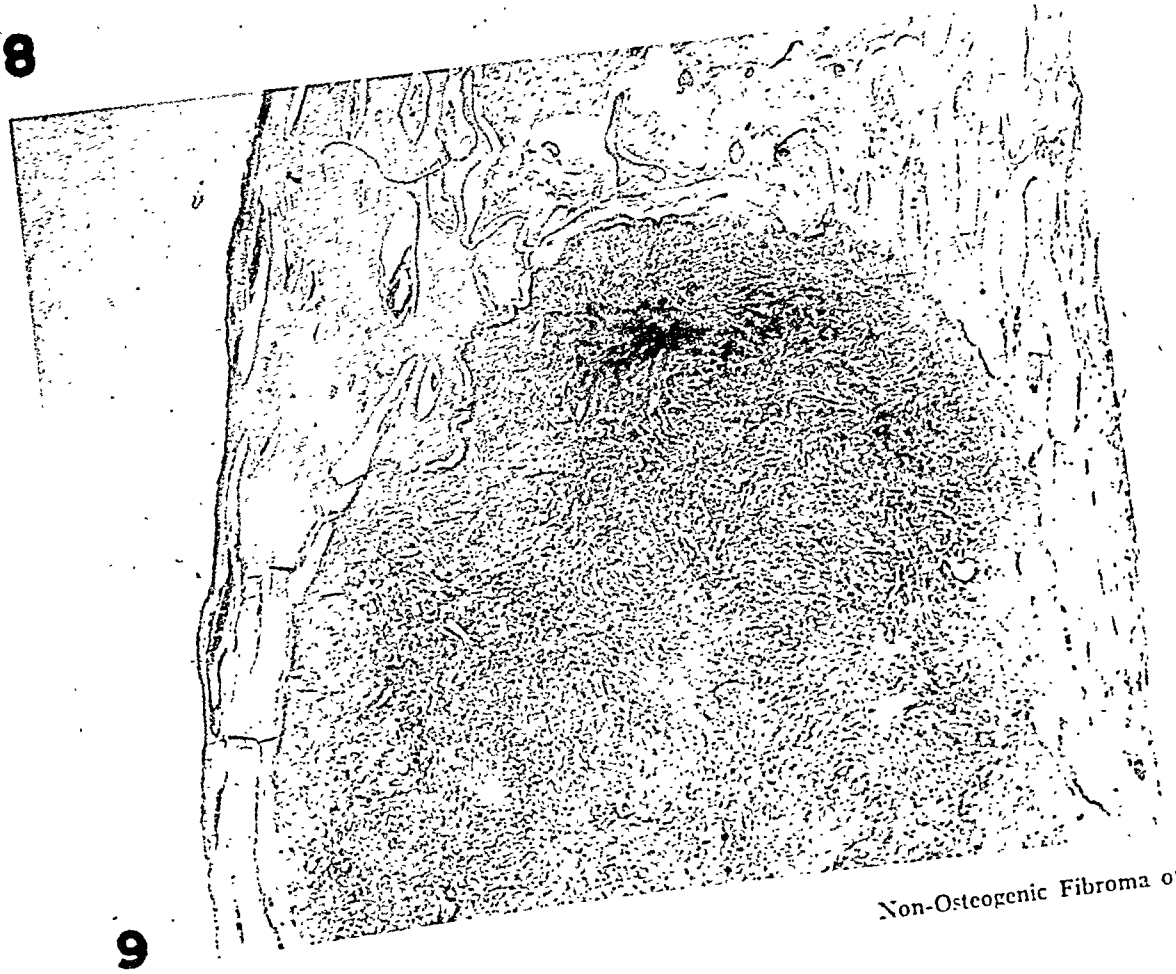
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7



8



9

Non-Osteogenic Fibroma of B

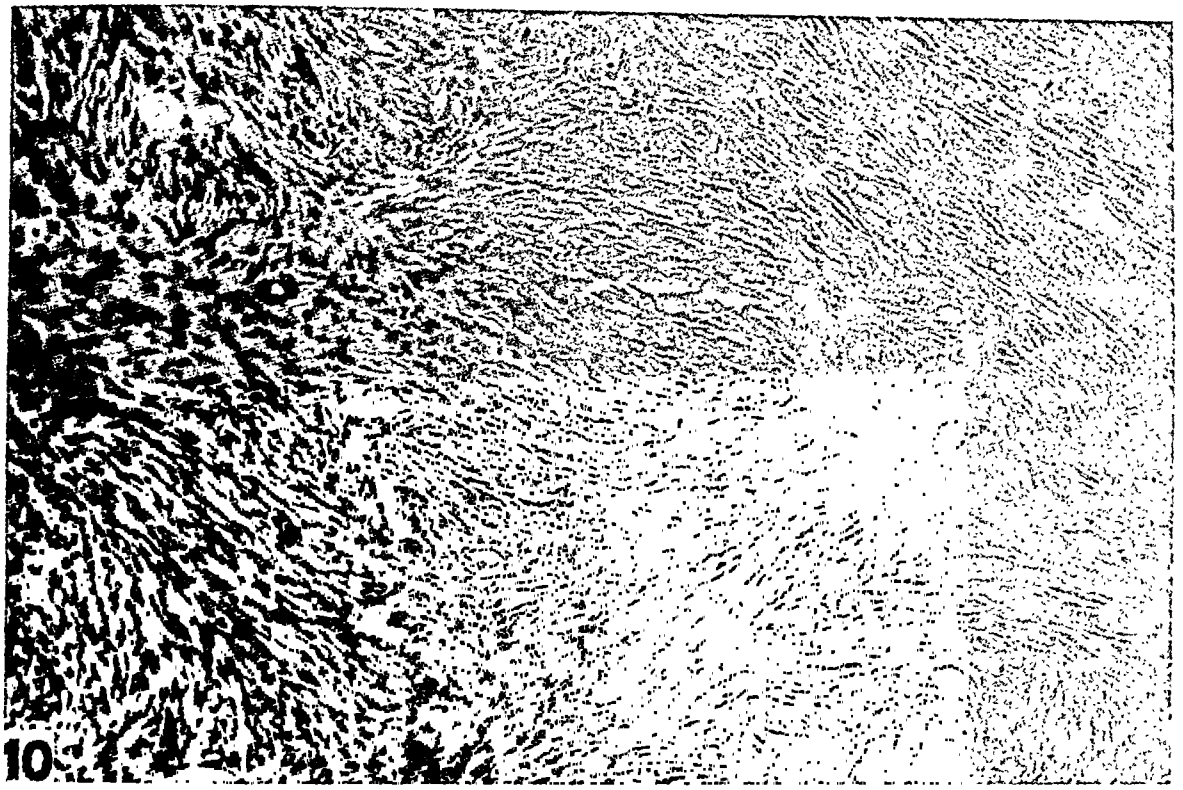
Jaffe and Lichtenstein

PLATE 33

FIG. 10. Photomicrograph showing the cytologic pattern of the tissue foci in the medullary cavity of the resected portion of the fibula shown in Figure 7. A whorled arrangement of the closely compacted small spindle-shaped connective-tissue cells is seen, with scattered small multinuclear giant cells interspersed among them. Osseous trabeculae are absent. × 200.

FIG. 11. Photomicrograph showing in greater detail the pattern presented by Figure 10. Both the stromal connective-tissue cells and the giant cells are small. The difference in the stroma-giant cell pattern in non-osteogenic fibroma from that in a giant cell tumor under the same magnification can be seen by comparing this figure with Figure 1 of our article on giant-cell tumor.[3] × 450.

FIG. 12. Photomicrograph showing an area in a non-osteogenic fibroma in which some stromal cells are undergoing, or have undergone, conversion into foam cells. However, in half of the cases discussed in this paper no such foam cell areas were seen. × 200.







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Non-Osteogenic Fibroma of Bone

BRENNER TUMOR OF THE OVARY*

CASE REPORTS, DISCUSSION AND BIBLIOGRAPHY

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Fritz Brenner (1907) is given credit for the original description of the tumor which bears his name, although he failed to recognize its true nature, as did at least nine others before him. He called it "oophoroma folliculare," and thought he was dealing with a form of granulosa cell tumor. Von Mengershausen (1895), Orthmann (1899), Gottschalk (1899) and Amann (1899) all described ovarian neoplasms which, judging from their descriptions, were undoubtedly Brenner tumors. Macnaughton-Jones reported a "solid ovarian tumor" in 1898, with illustrations showing that it belongs in this group. Orthmann designated his first two cases "adeno-fibroma cysticum carcinomatosum" and "fibroma papillare superficiale carcinomatosum," believing them to be forms of ovarian carcinoma, but called his third case "adenofibroma colloides ovarii." Schröder (1901), Lönnberg (1901), Gottschalk (1902) and Voigt (1903) reported tumors of this type under a variety of names. Voigt's case, and Orthmann's third case (which he considered to be benign) are of particular interest because the tumors involved both ovaries.

Ingier (1907), in the same year as Brenner, also described two cases. It is interesting that she called them "folliculoma ovarii," a term very similar to the one Brenner had used, although they worked independently. Ingier's cases were both bilateral, but she failed to comment further on this point.

It is noteworthy that four of the first seventeen tumors (23.5 per cent) which appeared in the literature were bilateral, because it indicated a much higher incidence of bilateral involvement than has been generally realized. Dockerty and MacCarty (1939) reported ten cases, none of which was bilateral. They stated that "95 per cent of all Brenner tumors are unilateral." All fourteen of the cases reported by Novak and Jones (1939) were unilateral. Proescher and Rosasco (1936), in reviewing the literature, stated that they were able to find only one bilateral occurrence, that of Weinzierl (1933). At the New York Post-Graduate Hospital, two bilateral cases were recently encountered among our surgical specimens in a period of 10 days. A review of our file since 1933 revealed two additional cases, one of which

* Received for publication, August 30, 1941.

was bilateral. Therefore three of our four cases (75 per cent) involved both ovaries. In this review all of our fibromas and granulosa cell tumors of the ovary were restudied; the former because the fibrous tissue might easily obscure the epithelial elements, and the latter because Brenner classified his cases with granulosa cell tumors. It is impossible to determine how often bilateral tumors occur because, in more than 50 per cent of the reported cases the opposite ovary was either not mentioned or microscopic examinations were not done.

Reports of Cases

Case 1 *

A white, married housewife, 42 years of age, was admitted to the New York Post-Graduate Hospital (F81419) in January, 1933, because of metrorrhagia and menorrhagia of 2 months' duration. Menses began at the age of 13 years and were "always regular." She had borne three children, and had had no miscarriages or operations. Physical examination revealed a firm, non-tender tumor of grapefruit size, in the right lower quadrant; the uterus was enlarged to the size of a 4 months' pregnancy, and displaced anteriorly and to the right. Supravaginal hysterectomy and bilateral salpingo-oophorectomy were performed.

On gross examination the uterus was found to be enlarged to 13 by 13 by 10 cm.; it was irregularly lobulated and there were several subserous, intramural and submucous fibromyomata. The fallopian tubes were grossly normal, but microscopically there was mild chronic inflammation. One ovary measured 3.5 by 2.5 by 1.2 cm. On section, numerous round, yellow areas up to 0.7 cm. in diameter were found in the stroma, as well as a thin-walled cyst containing clear fluid. The other ovary measured 3.8 by 2.0 by 1.8 cm., and on section there were round, yellow areas, up to 0.4 cm. in diameter, similar to those in the first ovary. In addition, there was an irregular, yellow, opaque area, situated beneath the surface, in the center of which was a minute cyst.

Microscopic examination† of the ovaries showed numerous corpora albicantia and sclerotic blood vessels. In a portion of each ovary there were localized areas of unusually dense stroma, in which numerous small, single and confluent islands of closely packed oval cells with oval granular nuclei were seen. In Dr. Meeker's original report, it was stated that "some of the nests were connected with strands of cells extending from the surface of the ovary," but this cannot be confirmed now because some of the original sections are no longer available. In one ovary the islands were composed of solid collections of cells, and in the other there were central cystlike spaces containing mucinous material and

* This case was presented by Dr. L. H. Meeker to the New York Pathological Society on April 23, 1936, and published in the Proceedings of that organization for 1935–36.

† Sections were stained with hematoxylin and eosin, Mallory's phosphotungstic acid-hematoxylin, Mayer's mucicarmine and Foot's modification of Hortega's silver carbonate stains for reticulum.

desquamated cells. The cells around these "spaces" were columnar and arranged in radial fashion (Figs. 1 and 2). A few islands had poorly outlined borders, while others formed somewhat indefinite elongated strands of epithelium. In one ovary some of the epithelial nests were situated in the thin wall of the cyst described above. This cyst was lined by pseudo-stratified, flattened columnar epithelium (Fig. 3).

Case 2

A negress, 39 years of age, was admitted to the New York Post-Graduate Hospital (J32113) on September 20, 1940, complaining of pain in the right lower quadrant and back for 3 weeks. Menarche occurred at 14 years, and her menstrual periods were always "regular." She had three "miscarriages," but no children. Subtotal hysterectomy was performed in 1936 at another hospital, for fibromyomata uteri. In 1937 and 1938, operations were done for "intestinal obstruction." There had been no menses since the first operation. On examination pelvic masses were palpated on the right side, and interpreted as a cyst of the broad ligament and a tubo-ovarian abscess. Bilateral salpingo-oophorectomy and appendectomy were performed.

One ovary, 4 by 1.5 by 0.5 cm., was pale yellow, and contained a firm nodule, 1 cm. in diameter, as well as several small, blue cysts. On section, the cut surface of the nodule was gray with faint tan areas. The other ovary, 4 by 2 by 1.2 cm., also contained a firm nodule at one pole, 2 cm. in diameter. On section, the cut surface of the nodule was homogeneously gray and generally similar to the nodule in the first ovary. The appendix and oviducts were grossly normal.

Microscopically, the nodules were composed of abundant dense, interlacing strands of fibrous connective tissue, in which there were numerous large and small nests of epithelial cells. The nests were sharply circumscribed, and the cells closely packed together. The latter were round to oval with vesicular cytoplasm and relatively large, oval, pale-staining nuclei. The nuclei contained finely granular chromatin and small distinct nucleoli. No mitotic figures were seen.

Case 3

A white female, 45 years of age, was admitted to the New York Post-Graduate Hospital (J52388) on September 29, 1940. She had had an abdominal tumor since 1931, which was gradually increasing in size. Menarche occurred at 14 years; the menses were always regular, with no unusual symptoms. One pregnancy in 1933 ended in spontaneous abortion after 2 months. Physical examination revealed bilateral ovarian tumors. Both ovaries, the right fallopian tube and the appendix were removed.

On gross examination the right ovary formed a lobulated mass, 13 by 10 by 7 cm., with oviduct attached. The external surface of the ovary was smooth and glistening, with numerous small blood-vessel markings. On section, there were numerous lobules from 0.5 to 6.0 cm. in diam-

eter. Each lobule had a dense, firm, tan and gray, fibrillary surface. A corpus luteum, 0.3 cm. in diameter, was also present. The oviduct was unaltered. The left ovary was similar to the right and measured 4 by 3.5 by 2.5 cm. On section, numerous lobules, similar to those described in the other ovary, were found, as well as a small serous cyst, 1 cm. in diameter. The appendix was 6 cm. long. In its distal one-third there were several firm nodules measuring up to 3 mm. in diameter which occluded this portion of the appendiceal lumen.

Microscopically, the ovaries revealed numerous small and a few large, well circumscribed nests of epithelial cells. The latter were polyhedral and of uniform size. The appearance was not unlike that seen in carcinoid tumors of the intestinal tract (Fig. 4). The nuclei were deeply stained and the chromatin material was in the form of fine granules. No intercellular bridges were identified. Some of the larger nests contained small, clear, cystlike spaces. The surrounding stroma was profuse and relatively avascular. It consisted of dense interlacing connective tissue strands and fibrils. The nodule in the appendix was composed of large strands and small nests of oval, vesiculated cells with pale-staining, round granular nuclei. It is worth noting that intracellular deposits of silver were demonstrated in the appendiceal nodules and not in the Brenner tumors by the silver impregnation method of Foot. The diagnosis was Brenner tumor of the ovary, bilateral, and carcinoid tumor of the appendix.

Case 4

A white housewife, 34 years of age, was admitted to the New York Post-Graduate Hospital (J24624) on September 21, 1937, complaining of dysmenorrhea for 21 years, which had been worse during the past 6 months. She had had one child, 11½ years before, and no miscarriages. The pregnancy was accompanied by swelling of the face and ankles. Menarche occurred at 13 years; menses were always regular at 28-day intervals, lasting for 6 days. The periods were usually accompanied by moderately severe "intestinal cramps." On physical examination masses interpreted as cystic ovaries were palpated in both lower quadrants. Salpingo-oophorectomy was performed on the right side and partial oophorectomy on the left.

Macroscopically, the right ovary was cystic and measured 4.5 by 4 by 3 cm. On cut section there were several corpora lutea and a firm rounded fibrous mass 10 cm. in diameter. The oviduct was grossly normal. The portion of left ovary measured 3.5 by 2 by 1.2 cm., and contained several small cysts and an atrophic corpus luteum.

Microscopically, the fibrous mass in the right ovary was typical of Brenner tumor. It was composed of a dense cellular stroma in which there were nests of varying sizes composed of epithelial cells. The cells were polyhedral, resembling squamous cells, and had small, round to

oval, faintly vesicular nuclei. In some nests there was a central cavity. The portion of left ovary contained a corpus luteum, as well as several corpora albicantia.

Histogenesis

Brenner (1907) thought that his cases were varieties of granulosa cell tumors. Meyer (1932), however, distinguished the tumor from those arising from granulosa cells on both morphologic and physiologic grounds. Meyer divided the known cases into two groups: (a) solid Brenner tumors with or without cysts, and (b) cystomata with Brenner tumors adherent to the cyst wall, with or without epithelium characteristic of pseudomucinous cysts. The four cases reported here probably belong in the first group, although a cyst was found in one ovary of case 1. It was Meyer's opinion that the tumor arises from the sexually indifferent cell inclusions first described by Walthard (1903). Walthard rests, as they are now called, occur not only in the ovaries, but also in the wall of the fallopian tubes and broad ligaments. It should be noted, however, that Brenner tumors have not been reported in the latter locations. According to Novak and Jones these nests may consist of "cells resembling squamous epithelium, often showing cystic hollowing, and perhaps actually converted into tiny cysts. On the other hand, the inclusions may take the form of glandlike spaces lined by columnar, perhaps ciliated, epithelium, with at times definite mucoid activity." In other words, Walthard rests may exhibit all the characteristics which have been observed in Brenner tumors, suggesting a close relationship of one with the other.

Schiller (1934, 1936) was struck by the resemblance between the transitional character of the epithelium in some Brenner tumors and the pathologic picture of cystitis cystica. He suggested that in some cases the tumor may arise from cells related to the epoophoron and rete ovarii. Novak objected to this theory on the ground that no relationship between these structures had been demonstrated.

The view that Brenner tumors represent one-sided development of teratomas, because of "their frequent association with pseudomucinous cystadenomas," was mentioned by Dockerty and MacCarty.

Plaut (1933) contended that Brenner tumors may arise from inclusions of the germinal epithelium in the ovarian stroma.

Incidence

Meyer, in 1932, was able to collect only 22 cases, including 4 of his own. Bland and Goldstein, in 1935, in an exhaustive study of both granulosa cell and Brenner tumors, found only 66 cases of the latter

variety. Varangot, in 1937, collected 108, and the following year Novak and Jones added 14 new cases, bringing the total to 122. I have been able to find 166 cases, to which I add 4 more. Of these, 97, or 57 per cent appeared in the last 10 years. The majority were unilateral; only 10 were bilateral, including a questionable case of Frankl's (1927). The 3 cases reported here bring the total number of bilateral cases to 13.

Clinical Aspects

In Table I the cases are classified according to age: 79, or 46.5 per cent were 51 years of age or over, and 41, or 24.1 per cent were between 41 and 50 years. In respect to location, 38.9 per cent of the tumors occurred on the right side and 33.5 per cent on the left; 7.6 per cent were bilateral, and in 19.2 per cent the side was not stated. The youngest patient was 20 years and the oldest 77 years of age.

The growth of a Brenner tumor is not attended by any recognizable group of symptoms. Uterine hemorrhage occurred in less than one-fourth of the cases. A "tumor in the abdomen" was the most common complaint. A considerable number were found accidentally, either at operation or at necropsy. Unlike the granulosa cell tumor, the Brenner

Table I

Classification of Patients with Brenner Tumors According to Age and Site of Tumor

Age in years	Right ovary only	Left ovary only	Bilateral	Side not stated	Total
0–10	0	0	0	0	0
11–20	1	0	0	0	1
21–30	5	3	2	1	11
31–40	14	3	2	7	26
41–50	14	18	3	6	41
51–60	17	20	4*	6	47*
61–70	11	12	1	3	27
71–80	5	0	0	0	5
Not stated	0	1	1	10	12
Total	67	57	13*	33	170*

* One questionable case.

tumor secretes no hormone and consequently produces no alterations in sex characteristics.

Morphology

Brenner tumors may vary in size from very minute nodules to as large as a man's head. Giles reported a case in 1909 as "adenocarcinoma?" in the wall of a multilocular cyst, which weighed 18 pounds; Davison and Neiman (1934) described a Brenner tumor of the solid type which weighed 15 pounds. The neoplasm is slow-growing and only rarely reaches large proportions. Grossly, the solid variety, which con-

stitutes about 70 per cent of all Brenner tumors, resembles a fibroma. On section, there is frequently a yellow tint to the cut surface. Gaines (1936) stated that "under magnification with the hand lens, small cavities may occasionally be discerned, varying from pinhead to cherry size, and containing an opaque, viscid, yellow brown fluid." The second type consists of a solid tumor in the wall of a cyst, usually a pseudomucinous cystadenoma. These tumors fall into Meyer's classification under groups (a) and (b), respectively.

Microscopically, the solid tumors are composed of abundant fibrillary connective tissue in which small nests of compact, polyhedral epithelial cells are embedded. The cells frequently become stratified and bear a strong resemblance to squamous cells. They may be cuboidal or cylindrical, but very rarely ciliated. Mitotic figures are not observed. Novak emphasized the presence of the characteristic nests of epithelial cells and the fibromatous connective tissue groundwork in combination and stated that both must be present to justify the diagnosis of Brenner tumor.

In most tumors there is some tendency, however slight, to central cystic degeneration of the epithelial nests. The superficial layer of the cystic portion frequently assumes a columnar character with mucoid secretion similar to that which occurs in pseudomucinous cystadenomata. It is this feature which helps differentiate a Brenner tumor from a granulosa cell neoplasm. On the other hand, granulosa cell tumors contain lipoid material which is entirely absent in the Brenner neoplasm. In addition, Brenner tumors contain glycogen (Meyer, and von Szathmáry) which is not found in granulosa cell tumors.

With the Foot silver stain the fibrillary character of the stroma is clearly demonstrated. The whorls and strands of connective tissue extend up to, but do not penetrate, the basement membrane surrounding the epithelial nests. The cells within the limits of the basement membrane are completely devoid of silver granules.

Novak and Jones agreed with Meyer's view that "at least a certain though small proportion of pseudomucinous cysts have their origin in Brenner tumors." Meyer also stated that some serous cystadenomas may have a similar origin, though no cases of this type have been observed.

Brenner tumors are benign lesions and no recurrence or metastases have been reported. A case reported by Tavildaroff was said to have recurred in the other ovary after a few months (Mandelstamm, 1932). Undoubtedly this was not a recurrence but merely another example of a bilateral growth, which either was not recognized at the initial operation or grew after the first ovary was removed.

Summary and Conclusions

Four cases of Brenner tumor of the ovary are reported, three of which were bilateral. A review of the literature revealed 166 cases previously reported under a variety of names. Only ten of these cases (6.0 per cent) were bilateral, including one doubtful case of Frankl's. Bilateral occurrences do not indicate metastasis. The tumor constitutes a distinct entity and is not to be confused with granulosa cell tumors.

The histogenesis of the Brenner tumor is not known, although there seems to be some relationship to Walthard rests. The use of the name "Brenner tumor" is recommended until it can be classified on a histogenetic basis.

Note: I am indebted to Dr. L. H. Meeker for the specimen and slides of the first case, and to Dr. S. M. Rabson for the translation of many of the foreign publications. I also wish to thank Drs. T. H. Russell, W. T. Dannreuther, and H. D. Furniss for permission to use their cases.

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DESCRIPTION OF PLATE

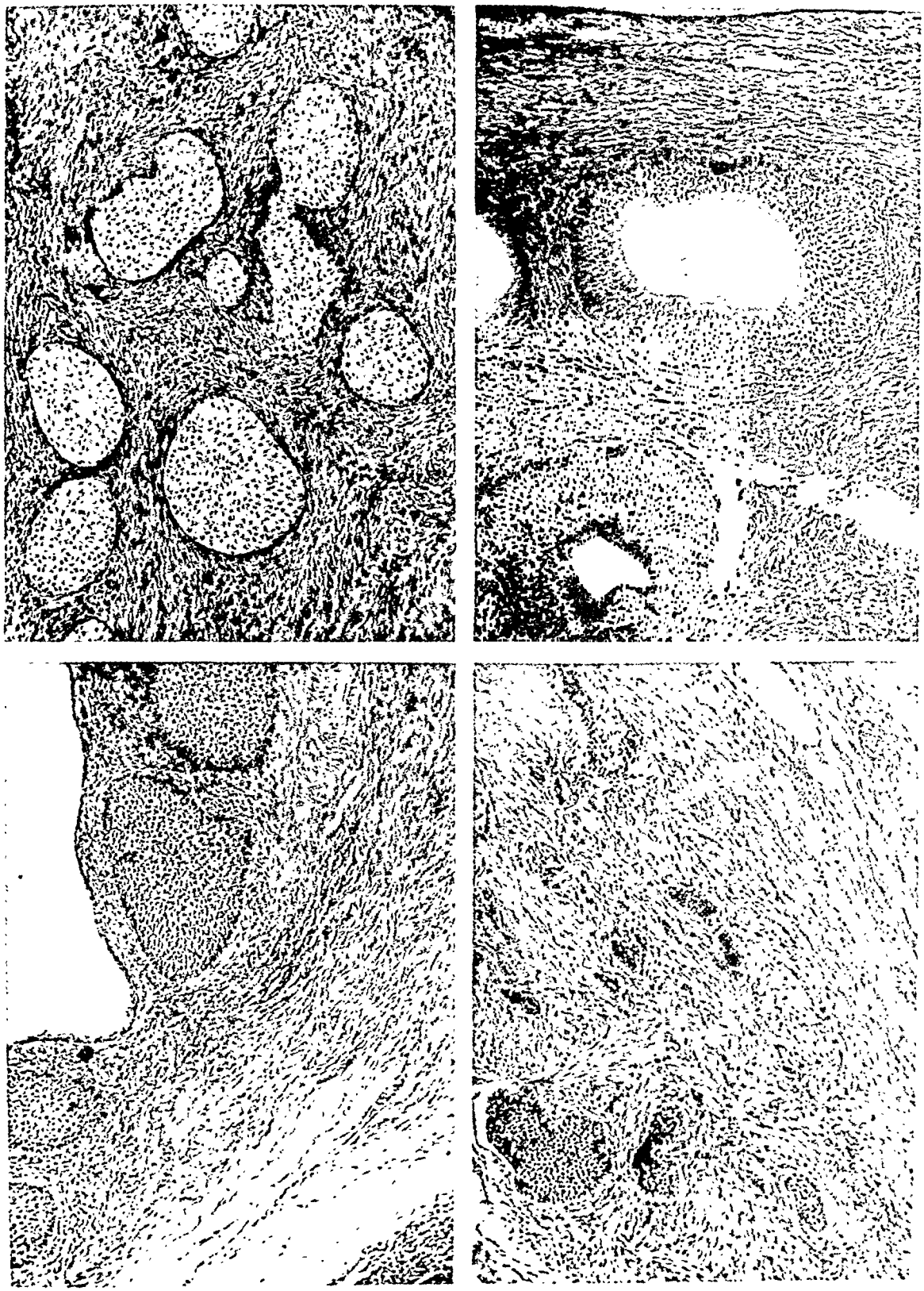
PLATE 34

FIG. 1. Case 1. Brenner tumor of ovary showing solid nests of cells in dense fibrous tissue stroma. × 82.

FIG. 2. Case 1. Cystlike spaces in nests of cells. The inner cells exhibit a radial arrangement and are columnar. × 82.

FIG. 3. Case 1. Nests of tumor cells in the wall of a large cyst. × 82.

FIG. 4. Case 3. Brenner tumor with superficial resemblance to carcinoid tumors. × 82.



Fox Brenner Tumor of the Ovary

STUDIES ON AMEBOID MOTION OF MOTOR NERVE PLATES*

II. Pathologic Effects of CO_2 and Electricity on the Explosive Ameboid Motion in Motor Nerve Plates in Intercostal Muscle

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The explosive structural changes produced in motor nerve plates and related muscle striae by either strong carbon dioxide or electrical stimulation are unknown. Evidence has been presented which casts serious doubt on the prevalent opinion that the nerve plate in a mature and specific striped muscle fiber remains constant in size, shape and internal structure during the alternate phases of contraction and relaxation, and in contracture or rigor.[1]

Evidence is likewise presented in this paper which casts serious doubt on the prevalent concept that muscle fibers are permanently fixed into two types; namely, coarsely and finely striated. The manifold forms of the motor nerve plates and structural variations of the related muscle cross striations defy any simplification by a static morphologic classification. An understanding of the dynamics of functional ameboidism of the motor nerve ends and the influence of the changeable capillary chemistry in producing the different periodic structural changes in striped muscle may help to clarify this complex problem. At any event, only photographic evidence that others may evaluate of structural changes in muscle and nerve is of importance in the present state of confusion. Free-hand drawings and verbal descriptions have not clarified the problem.

Some of the recent excellent reviews that demonstrate the complexity of the problem which exists in reference to the normal structure of either motor nerve ends or muscle have been made by Cobb,[2] Needham,[3] Hines,[4] Jordan,[5] Hinsey [6] and Zeit.[7]

It is firmly established that protoplasmic movement, or ameboidism, occurs during the initial growth and during the regeneration of nerves. Ameboid movement at synapses during functional activity of the nervous system was assumed by Cajal.[8] He discovered that ameboidism was a reality by the gradual changes of position of growth cones of both embryonic and regenerating nerves. This movement was estab-

* These investigations were carried out with the aid of grants for research to the Department of Anatomy of the Marquette University School of Medicine by the Committee on Scientific Research of the American Medical Association, and the National Foundation for Infantile Paralysis, Inc.

Received for publication, July 11, 1941.

lished by the study of progressive changes in position of growing nerve terminals in fixed material. Observations of the actual protoplasmic movement in the growth of living embryonic nerves in tissue culture were made first by Harrison,[9] then by Lewis,[10] and were confirmed by numerous other workers, especially recently by Speidel.[11]

Evidence was presented for a rhythmic alternation of the dilatation and retraction of the motor nerve plates during the mature functional activity of intercostal muscle.[1] In the fractional contraction of muscle the resting or relatively relaxed fibers were characterized by retracted plates associated with coarse, widely spaced cross striations. The strongly contracted intercostal muscle fibers during hyperpnea by carbon dioxide had dilated nerve plates and fine, closely spaced cross striations. There were many transitional stages between these two extremes. The surface area of the motor nerve plate was increased during contraction of the muscle fiber. By physiologic methods Cooper [12] studied the relation of active to inactive fibers in the fractional contraction of muscle.

Evidence has been presented that the dark, condensed nodes and the light, rarefied internodes of cross striations in fixed striped muscle in heat rigor are the two structural expressions of a "frozen-standing" system of longitudinal compressional waves. These mechanical pressure waves accompany the strong capillary chemical changes within the muscle, and are expressed as periodic bands when the working muscle substance is confined and enclosed in an elongated, microcapillary space of relatively constant volume. The variable number of these cross bands is directly related to the variable speeds, concentration and composition of the reversible or irreversible capillary chemical reactions. When the speed of capillary chemical change, influenced by temperature, is at an optimum high, there is a greater number of cross striations in the same muscle fiber than when the speed is low. The explosive compressional waves that accompany the capillary chemical changes in the normal microcapillary muscle fiber determine the structural arrangement of the protoplasm, in space, into zones of condensation and rarefaction.[13]

Buchthal [14] stated that "The activity of a muscle in every respect is an interference phenomenon, and it is only possible to make a real analysis of the mechanical and electrical response of the muscle, and also of its optical properties in relation to its structure, if one works with single muscle fibres. The more we know about the minute structure of the fibre, the more we shall know about the mechanism of a muscle's ability to vary its length and tension."

Presumptive evidence that the motor nerve plates physically vibrate

was presented on the histologic basis of the radiation patterns of muscle striae related to the nerve ends.[15] More direct evidence of the physical vibration, or alternation of large and small size of the same nerve plate, was found in intercostal muscle from rats, the respiratory rate of which was accelerated by carbon dioxide.[1] The reappearance of the granular sole plate of Kühne surrounding the retracted axonal ramifications during relaxation and the disappearance of this granular material in the expanded axonal ramifications during contraction lends support to the chemical theory of impulse transmission. The periodic appearance and disappearance of the granular sole plate of Kühne lends support to the theory that there is a substantial transmission, rather than that of a more subtle electric current, from the motor nerve end to the muscle. This experimental histologic evidence, therefore, lends support to the chemical theory of conduction in nerve to muscle at the myoneural junction.

These structural changes during function also give support to the very recent theory of Rosenblueth [16] that the nerve plate in muscle is a chemical secretory microgland. This morphologic evidence of the explosive periodic change in the structure of the nerve plate and muscle in functional activity likewise favors the recent "trigger concept" of the rhythmic explosions in muscle proposed by Fenn,[17] as follows: "Thus at the desire of the experimenter a whole series of new chemical reactions can be thrown into activity in an orderly and reproducible manner. This sudden transformation so produced may be properly described as an explosion. The muscle is a self-cocking explosive machine with a convenient trigger. The explosion turns chemical potential energy into mechanical energy . . . The forces developed into a muscle 'explosion' are of amazing magnitude." Sacks [18] concluded his excellent review on the chemistry of muscular contraction by this final pertinent statement: "Finally, and most elusive of all, there remains the question of the mechanism by which the muscle transforms the chemical energy derived from oxidative or anaerobic reactions, into the mechanical work which is its function in the body."

Hines [19] stated that the nerve ends in striped muscle are "morphologically lawless." Wilkinson's [20] developmental theory was proposed to explain the morphologic variations in motor nerve plates in striped muscle. He wrote:

"These series of figures seem to indicate the probability that at least some of the *terminaisons en grappes* are immature forms of somatic motor plates and, morever, illustrate the various stages in the development.

"It is necessary to point out, however, that although the grape-like endings are here regarded as immature motor endings, it is not implied that they will necessarily eventually develope during the life of the animal into plate-like endings. Whether

they are endings which represent a reserve and can under certain conditions develope into plate-like endings or are endings in which development is definitely arrested, cannot be stated with any certainty. All that can be said, is that these grape-like endings can be arranged in a series which shows an increasing degree of resemblance to plate-like endings, and for that reason are here called immature motor endings. The condition of affairs, however, found in mammalian embryonic material and in young mammals and also in material from nerve regeneration experiments, seems to lend support to this conception."

Since the periodic changes in the size, shape and internal structure which occur during the normal functional activity of a specific motor nerve plate are unknown, it is not surprising that the sudden and explosive pathologic structural changes in nerve and muscle produced by strong stimulation with carbon dioxide and electricity are likewise unknown. Relatively chronic changes in motor nerve plates produced by starvation (Denny-Brown [21]), dietary deficiency in beriberi (Woollard [22]), and nerve degeneration (Hines,[19] Cajal,[23] Tello [24] and numerous others) are well known.

It is appreciated that for absolute conclusiveness, there is one weak link in the chain of evidence for the periodic motion of motor nerve plates, and this is the lack of an actual cinematographic record of the rhythmic expansion and retraction of one motor nerve plate during the functional activity of its muscle fiber. It is hoped that the technical difficulties involved in this problem will soon be solved. However, Cajal,[8] 50 years ago, came to the conclusion that ameboid motion lay at the basis of the growth of nerves, and produced evidence derived from fixed tissues before the motion picture was used in science. Very little imagination is needed to visualize the periodic changes in the motor nerve plates in relation to the cross striations of the muscle fiber when adequate photographic evidence is available.

I am familiar with no photographic evidence in the literature wherein the morphology of the nerve plates and of related muscle striae are both presented in continuity under normal and abnormal experimentally controlled conditions in the same animal. Kühne's [25] exhaustive comparative study and excellent paper with 346 drawings presented the structural differences in the nerve plates in different species of animals, but it ignored the related muscle structure. He neither presented evidence, nor reached the conclusion, that functional ameboidism was the underlying factor of the pleomorphism of the nerve plates and of their effect on the muscle striae in one muscle.

The purpose of this paper, therefore, is to present conclusive photomicrographic evidence in the form of a permanent atlas of the normal and abnormal pleomorphism of motor nerve plates due to functional ameboid motion. The immediate explosive changes in the size, shape

and internal structure of motor nerve plates in the right intercostal muscle of the same animal (white rats nos. 25 and 57) are produced as explosive effects of carbon dioxide and electrical stimulation. Fortunately the apparently abundant photomicrographic evidence with legends is clear enough, without a long detailed morphologic description; and this objective evidence is needed at the present time in order that others may evaluate it and make their own interpretations. The exact and clear presentation of easily verified facts necessitates the large number of illustrations in view of the contemporary state of confusion regarding the significance of the structural variations of the motor nerve plate and the related muscle cross striations in the physiologic and pathologic motions of muscle with its innervation intact.

Materials and Methods

Rats were anesthetized by the intraperitoneal injection of nembutal, 25 mg. per Kg. The hair was clipped with the electric clipper over the right dorsal aspect of the thorax. A 1 inch vertical incision, 1 cm. to the right of the midline of the spine, was made through the skin and muscle down to the ribs. The sixth, seventh and eighth ribs and related intercostal muscles were quickly excised in one piece beginning medially 1 cm. from the spine and extending 1.5 cm. laterad.

This excised part of the thoracic wall was run through the gold chloride technic and served as a control of the relatively normal motor end-plates in the intercostal muscles of the right side. The bleeding was quickly controlled by hemostats and ligatures. The skin was sutured over the thoracic gap and the animals immediately placed for 1 to 10 minutes in a jar, the air of which contained roughly 15 to 30 per cent of carbon dioxide. The rats were removed from the jar and a second quick excision of the right second, third, fourth and fifth ribs and attached intercostal muscles of the right thoracic wall was made. This excised portion was at once run through the gold chloride technic for microscopic study of the teased muscle fibers and motor end-plates. After again suturing the skin, animals numbered 1 to 25 were allowed to breathe normal air for a period of 3½ hours, and then were decapitated. In another group of rats (nos. 26 to 75) the same procedure was carried out except that the animals were killed immediately by electrocution instead of decapitation. This was done immediately after the second excision of the right second, third, fourth and fifth ribs and attached intercostal muscles of the right thoracic wall. The rats were suddenly killed by electric stimulation for 1 minute, through the spinal cord, with one electrode in the cervical region and the other in the lumbar region. The ordinary alternating current of 120 volts, 1 ampere,

was used. The ninth, tenth and eleventh right intercostal muscles were then excised, subjected to the gold chloride technic and teased.

The motor end-plates in the intercostal muscle, fixed after different rates of respiration, in the white rat (*Mus norvegicus*), were stained by the Bielschowsky method of silver impregnation as modified by Boeke;[26] by the *intra vitam* methylene blue method of Ehrlich as modified by Huber [27] and by Huber and DeWitt;[28] and by the Ranvier gold chloride method as modified by Wilkinson [20] and by me. The continuity of the medullated nerves, motor nerve plates and related muscle structure is best studied in teased muscle fibers after the following gold chloride method:

The muscles are cut with a sharp surgical scissors into pieces about 3 to 5 mm. thick and fixed in filtered, fresh, full-strength lemon juice. After 10 to 15 minutes, the lemon juice is decanted and a 1 per cent solution of gold chloride poured over the tissue without any washing in distilled water. (It was found that better results were obtained when the gold chloride solution was made up a day or two before use.) The bottle containing the metallic salt and tissue is kept in the dark until the bits of muscle assume a uniformly golden appearance. The time during which this is accomplished will vary from 10 minutes to 1 hour, so the only criterion that can be depended on for sufficient impregnation is the color. If left in the gold chloride solution until brown, the technic will prove to be unsuccessful. Without washing in distilled water the tissue is transferred to a mixture of one part of formic acid to three parts of distilled water and placed in the dark for 12 hours. (In the usual methods described, the muscle tissue remains in the acid solution from 24 to 48 hours. However, it was found by experience that when the tissue was cut thinly and uniformly so that just enough of the metallic solution penetrated the tissue evenly, the gold was nicely reduced and the muscle tissue was beautifully stained a reddish blue color with the nervous elements black, within 8 to 12 hours. If a longer period was required, it indicated a fault in technic in using the gold chloride solution, so that results were not good in any such case.)

After 12 hours in the formic acid solution, the pieces of muscle tissue are washed once in tap water and quickly placed in a mixture of equal parts of 50 per cent alcohol and chemically pure glycerin. This prevents the swelling that has been so often reported as occurring when the tissue was placed in pure glycerin.

A bit of tissue is removed, oriented in a drop of glycerin on a 1 by 3 inch slide with a pair of sharp teasing needles, and gently spread with a coverslip, care being taken not to do too much teasing with the needles so as not to disrupt the integrity of the muscle fibers with respect to each other and to their nerve endings. The slips are sealed to the slide with a ring of clarite, a nitrocellulose product far superior to the old paraffin. Many of my slides so mounted have kept for over 2 years without any noticeable deterioration. The difficulty with the old method has thus been obviated; namely, leakage through the paraffin ring allowing entrance to saprophytic organisms which produced decomposition beneath the slip.

Seventy-five rats were used in this study and over 5000 slides of teased muscle were made during the past 6 years. The motor nerve endings observed during this period would approach one million.

The Bielschowsky silver technic reveals the fine neurofibrillar, reticular nature of the fronds of the axon. The sarcoplasm of the sole plate

is clear and unstained by methylene blue. The gold chloride method, however, demonstrates the sole plate, when present, to be composed of fine granules. There is a narrow, clear area between the fronds or branches of the terminals and the granular muscle sole plate. In some expanded plates there is direct continuity between the serrated projections of the terminations of the axon and the dark muscle striae. The ovoid nuclei of the sole plate remain unstained by the gold method and form clear spaces surrounded by dark granules. The periterminal network of Boeke [26] is best seen in expanded nerve plates after staining by the silver method. It forms a foamlike reticulum between that of the nerve terminals in the plate and the periterminal network in the sarcoplasm of the sole plate. In the expanded nerve plates, which appear to be a chemical secretory apparatus, there is an apparent finer structural continuity between the terminal nerve plate and the surrounding muscle sole plate when stained by the silver method. In the retracted plates this apparent continuity is lost. The line of demarcation between nerve terminals and sarcoplasm is not a constant one. It depends upon the state of functional activity in which life is stopped and the tissue fixed. Although the structural integrity of both muscle and nerve is preserved at the myoneural junction, it appears that some chemical secretory granules pass from the nerve end to the muscle in some rhythmic manner.

The magnitudes of the muscle fibers are slightly enlarged by gentle compression and by the technic of preparation. The coarseness and fineness of the cross striations in the muscle fibers are in about the same relative proportions as during life in spite of the histologic technic. The continuous compression by the coverslip is counteracted by small supporting bits of coverglass. In photographing teased preparations of muscle fibers from 30 to 150 μ thick it is obviously impossible to bring simultaneously into focus all of the structures seen in any particular field. This difficulty, however, was partially surmounted in some photographs by making multiple exposures at different focuses on the same photographic plate. Careful observations were made to obtain records of those motor end-plates in which the cross striations were in the same focus as the related nerve plate. In variations of the focus there were changes in the pattern of muscle structures, especially in the thick fibers.

Experimental and Histologic Results

The respiratory rate after nembutal anesthesia in the white rats varied from 25 to 55 per minute. During excision of the piece from the right thoracic wall the rate increased to 60 to 80 per minute. This was

the reason for making the first incision through the ribs along the dorsal aspect, thereby severing the nerve supply to the excised muscles quickly and before the respiratory rate increased. These muscles were used as relatively normal controls. The rats were then placed in carbon dioxide for 1 to 10 minutes. The respiratory rate immediately was raised and varied from 130 to 200 per minute. When the rate of labored breathing fell below 100 per minute, the rats were taken out of the jar. The second excision of the right second, third, fourth and fifth ribs and attached intercostal muscles was quickly made. The respiratory rate fell to 60 per minute within 30 minutes while the rats breathed normal air, and remained at this rate until the animals were decapitated 3½ hours later. By this simple means the histologic changes in the same group of muscle motor nerve plates in the same animal (rats nos. 1 to 25) were observed under different degrees of intensity of functional activity stimulated by the chemical method of carbon dioxide.

In another group of rats (nos. 26 to 75) the above procedure was repeated except that the animals were killed by electrocution instead of by decapitation. This was done immediately after the second excision of the right third, fourth and fifth ribs. In this series of animals the effect of respiratory superactivity by carbon dioxide and the lethal effect of electricity were superimposed.

The motor nerve plates from the right intercostal muscles in the relatively normal controls, excised prior to carbon dioxide stimulation, varied from the retracted state with wide, short fronds 21 to 25 μ in diameter (on middle muscle fiber Fig. 2, on upper muscle fibers Figs. 3 and 4, and middle muscle fiber Fig. 7) to the expanded state with long, attenuated pseudopodia 52 to 55 μ in diameter (on lower muscle fibers Figs. 3 and 5). These were the extremes in the dimensions of 2000 normal nerve plates.

The normal and abnormal retracted motor end-plates with wide, coarse fronds are related to coarse, widely spaced cross striations (Figs. 1, 2, 3, 4, 7, 9, 11, 14, 22, 25, 32, 36, 40, 51 and 59). The expanded plates with narrow, elongated processes are related to fine, closely spaced cross striations (Figs. 1, 2, 3, 4, 5, 6, 8, 13, 15, 30, 31, 34, 35, 39, 50, 58, 63 and 64). This relationship of the retracted and expanded motor nerve plates to a different number of cross striations is clearly demonstrated in Figures 25 and 31.

There are 11 dark cross striations related to the retracted nerve plate in the relaxed muscle fiber (Fig. 25). There are 32 dark cross striations related to the expanded motor nerve plate in the active muscle fiber (Fig. 31). This differential numerical relationship of the muscle striae related to the retracted and expanded nerve plates is also related

to physiologic and pathologic functions at the myoneural junction. This finding confirms the classification of muscle fibers by Hines [19] into coarsely and finely striated. There was, however, a closely graded series of transitional stages in the structure of the muscle fibers between these two extremes with variations in the number of the cross striations in the muscle. This agrees with the observations reviewed by Cobb,[2] Needham,[3] Hines,[19] and Hinsey.[6]

The prepared fibers from the right intercostal muscle stimulated by carbon dioxide to superactivity had, in 2000 measurements, 673 plates that varied from 29 to 55 μ in diameter. The largest motor nerve plate expanded by carbon dioxide stimulation of the respiratory center reached the diameter of 112 μ (Fig. 50). In 2000 measurements of the diameters of the stimulated motor nerve plates, 1327 varied from 55 to 91.4 μ and had 2 to 7 elongated, attenuated, distinct processes with periodic enlargements. These thin processes were separated by wide meshes and were related to fine, closely spaced cross striations. In some places the protoplasmic projections of the nerve plates formed anastomoses, and on some muscle fibers there were two discrete motor nerve plates. These were the two terminals of the two medullated branches of a single axon.

The sole plate of Kühne formed a dark, granular rim around the retracted and slightly expanded axon of the normal motor plates (Figs. 4, upper muscle fiber; 7, middle muscle fiber; 9, upper muscle fiber; 11 and 14, upper muscle fibers; 25, 26, 27, 32, 36, 51 and 59). This granular sole plate of Kühne disappeared in the greatly expanded end-plates related to fine, closely spaced cross striations (Figs. 3, lower muscle fiber; 8, 13, 30 and 31). There was a reduction by 80 per cent in rats nos. 1 to 25 in the number of motor nerve plates expanded beyond 55 μ in diameter in the right ninth, tenth and eleventh intercostal muscles after the animals were again breathing normal air for 3½ hours. During this time the rate of the labored respirations fell from 200 or 100 to 50 or 60 per minute. In the superactive muscle fibers (Figs. 30 and 31) it is objectively evident that there is a substantial increase in size of the motor nerve plates. The clear ovoid areas both within the nerve plate and in the muscle substance are occupied by nuclei of the sole plate of Kühne.

The expanded nerve plates in the active muscle fibers have variable degrees of ameboid extensions of the terminal arborization of the axon. There appears to be a centrifugal extension of the terminal arborizations of the axon of the nerve plate, with increase of the surface area during muscle contraction; and a centripetal retraction of the processes, with decrease of the surface area, during relaxation of the muscle fiber.

Fractional contraction, in which some fibers are active, others at rest, appears to be the cause of these differences in structure. The active motor nerve plates have a greater number of dichotomous terminal divisions than the inactive ones. The nuclei of the granular sole plate of Kühne occupy the spaces between the divisions of the axon.

The diameter of the muscle fiber is not a reliable basis for classification of the muscle fibers (Figs. 22 and 23). Some wide fibers have coarse striations and some narrow fibers contain fine striations. This is in part dependent upon whether the muscle fiber is fixed in a state of isotonic or isometric contraction. In some fibers there is a simple alternation of dark Q and light J bands. The Q and J bands vary in width. In some there is a doubling of the Q band and in others the pattern of the so-called constant sarcomere structure of Z, J, Q, J, Z occurs. This pattern, however, is highly inconstant. There is, therefore, no constant number of so-called sarcomeres in this striped muscle.

These cross striations, therefore, in muscle, have variations in number and patterns such as the following: single Q bands (Figs. 2 and 3), double Q bands evenly spaced or increased in width in a geometric series radiating from the motor nerve plate (Figs. 3 and 8), J bands wider than the Q bands or the reverse (Figs. 17, 18 and 24), Q bands coarse (Figs. 6, 11 and 22), Q bands fine (Figs. 12, 13 and 23), Q bands arranged in a spiral, vernier shift or zigzag crossings (Figs. 2, 11 and 22), Q bands having a radiate pattern (Figs. 8 and 14), or the so-called sarcomeric pattern of Z, J, Q, J, Z (Figs. 12 and 23). There may be multiple patterns in a single muscle fiber (Figs. 8, 12 and 23). In cross sections there may be saturnine rings of the Q bands. It is readily apparent, therefore, that there are multiple variations of the cross striations in the intercostal muscle fibers. This may be confirmed at once by an inspection of the changeable patterns shown in Plates 1 to 18. There is no fixed pattern based on a unit of structure, the so-called sarcomere, in the cross striations of the intercostal muscle in the white rat. The internal structure of the intercostal muscle is as variable as its function.

There is dichotomous branching and anastomotic reticulation of the branchings of the axon in some of the nerve plates. In some plates there are terminal enlargements of the ramifications of the axon in the nerve plate. This enlargement of the nerve plate during increased functional activity is objective evidence of the transference of substance from the nerve to the muscle.

With progressive increase of ameboid expansion of the motor nerve plates there is a corresponding increase in number and fineness of closely spaced cross striations. The strongly contracted muscle fibers

have large motor nerve plates with two or more moniliform projections separated by wide spaces. With progressive increase in size of the motor nerve plate and increase of fineness of the dark and light transverse gratings, the contracted muscle fiber takes a lighter stain. There are many transitional stages and gradations between the coarsely and finely striated muscle fiber. There is a substantial ameboid protrusion of the processes of the nerve plate in various directions in the muscle substance which increases the surface area of the nerve plate. This is evidence that during life the muscle striae are not constant and rigid membranes.

There is an obliteration of the muscle striae below the plate on the lower muscle fiber shown in Figure 14, and a biconvex radiation of the muscle striae toward the left and right of the muscle fiber, away from the plate. These changes are likewise observed in nerve and muscle fixed *in situ* under normal tension by the terminal skeletal attachments. This pattern in the muscle is not one due to abnormal optical effects in photography, nor to crushing in teasing, nor to the pressure of the coverslip, since all these sources of error have been eliminated. This localized obliteration of the striations underlying the double motor nerve plate is observed at all levels of the focus. This motor plate is composed of two discrete components contributed by the dichotomous division of the single axon. There is a gradient in the fineness and spacing of the cross striations in the different phases of the functional activity of the muscle fiber in fractional contracture within a single muscle fiber.

When the motor nerve plate expands at one pole more than at the opposite one there is frequently a differential pattern of the related cross striations in a single muscle fiber (Figs. 15 and 18). At the upper, relatively non-expanded pole the striae are coarse and relatively regularly spaced. The oblong clear areas at the periphery of the nerve plates, in the granular sole plates of Kühne, are occupied by nuclei. There is a massive conduction of nerve substance into the axonal ramifications in the expanded motor nerve plates, stimulated by carbon dioxide.

The variable radiation and rhythmic replacement of the cross striations related to the functional activity of the motor nerve plate in the muscle fiber explains the differential morphology of the cross striations of the muscle (Figs. 16, 17 and 18). Irregular granular zones in the muscle fibers are related to motor nerve plates. The coarse striations at the ends of the muscle fibers are replaced by those related to an activity which appears to wipe out the striations underlying the nerve plates. This obliteration of the striations appears to occur at the

beginning of certain degrees of intensity of the propagated disturbance that radiates through the muscle fiber from the motor nerve plate. These are radiation areas of contracture in muscle, beginning at the nerve plate. Periodic areas of fine striations alternating with coarse ones are found in the muscle fiber (Figs. 19, 20 and 21). This subsequently results in a graded series of the spacing and width of the dark cross striations when fixed at the initial phases of expansion of the motor nerve plate in striated muscle. The pattern of the cross striations in muscle underlying the nerve plate therefore is highly inconstant.

The retracted plate (Fig. 32) has the clear ovoid regions occupied by the nuclei of the granular sole plate of Kühne close to the axonic nerve plate. With progressive explosive expansion of the plates by ameboid motion and sudden chemical changes these clear oval areas are projected into the muscle substance and at some distance from the nerve plate (Figs. 30, 33, 34, 35, 39, 48, 50, 51, 55, 58 and 63). There is a replacement of the clearness of the periodic structures in the muscle by the irregular granulation. The granules irregularly displace the cross striations. The ovoid clear areas in the muscle substance occupied by nuclei are surrounded by a dark granular radiation. In some places these clear oval areas may resemble vacuoles in both the nerve plate and the muscle.

Superactivity of hyperpnea evidently favors neurocladism, or dichotomous division of the branches of the axon in the nerve plate in intercostal muscle (Figs. 33, 34 and 35). The obstacles composed of nuclei of the sole plate of Kühne found in the crotch of the dividing terminal axon may condition the morphology of the expanding motor nerve plate during superfunctional ameboidism. There is a massive transmission of nerve substance into the axonal ramifications in the expanded motor nerve plates, stimulated by carbon dioxide. There is divisional ameboidism (Fig. 34) of the terminal axon, the projections of which grow out by superfunctional stimulation among and beyond the nuclei of the granular sole plate (Fig. 34).

These projections normally do not extend beyond the granular material of the sole plate of Kühne. The strong chemical, CO_2, acts as a neoformative stimulus to the production of the axonic branches of the motor nerve plate. There is a varicose or moniliform pattern to the axonic ramifications. When the axon is strongly stimulated and its terminals meet the resistance of nuclei and other protoplasmic obstacles, there results a precipitous and prolific projection of ramifications in the form of a bouquet (Figs. 8, 34, 35, 50 and 58). The dichotomous divisions extend between the nuclei of the dark granular sole plate of Kühne. The protoplasmic movement is the result of capillary

chemical change in concentration or composition, or both, of the axonic protoplasm, with a consequent modification of the internal pressure which extends along the lines of least resistance into the myoplasm.

It is this functional ameboidism of the terminal axonic bulbs that determines the pleomorphism of the motor nerve plates in striped muscle. It is evident that there is no constant morphology in the 88 motor nerve plates shown in Plates 1 to 17.

The explosive patterns in muscle and nerve are not artefacts due to compression and teasing of the muscle fibers. They are found likewise in muscle fixed intact in the animal and then serially sectioned. The best evidence is obtained, however, when the continuity of the motor nerve plate and muscle fiber is maintained by teasing only gently and by preserving them together by the gold chloride method.

It is noteworthy that, in the direction in which the nuclei are projected from the periphery of the nerve plate into the muscle substance, there is a streamlike appearance in the sarcoplasm and a displacement of the regular pattern of cross striations by an irregular explosive arrangement of granules (Figs. 43, 44, 45, 46, 48, 50, 52, 54, 55 and 58). There is a direct continuity by a stream-line of granules between the clear ovoid and fusiform areas in the muscle substance and the granules of the sole plate of Kühne surrounding the axonic branches of the motor nerve plate (Figs. 44, 45, 48, 50, 52, 55 and 58).

The ultra-terminal collateral is observed (Fig. 62) as a fine nonmedullated branch of the axonal ramification in the typical, expanded, somatic motor nerve plate, and it ends in the same muscle fiber a short distance from the plate of origin. There is a terminal collateral which arises from the axon a short distance before it penetrates the sarcolemma and which ends as an independent small plate above the main nerve plate (Fig. 61). The protoplasmic extensions of the nerve plate may be related to the expansive effect of a strong electric stimulation or of any other strong chemical or mechanical stimulus.

Upon strong electrical stimulation there is a complete explosive disruption, dissolution and granular replacement of the arborizations of the axons in the two neighboring motor nerve plates (Fig. 67). This condition is found in 0.5 per cent of the motor nerve ends. There is likewise a displacement of the periodic pattern of muscle striae in this area of sudden and explosive disappearance of motor nerve plates by granular disorganization through strong electric stimulation. The nucleated ovoid clear areas are irregularly scattered in the myoplasm surrounding the ghostlike outline of the motor nerve plates. There is a massive transportation of nerve substance into the axonal branches in the expanded motor nerve plates, stimulated by electricity (Figs.

60, 61, 62, 63, 64, 65 and 66). There is an accompanying explosive perturbation of the related protoplasm of the striped muscle fiber. The surface area of the strongly stimulated motor nerve plate (Fig. 63) is greatly increased over that of the relatively resting nerve plate (Fig. 59). This difference in size of the plates in the same stimulated nerves and muscle fibers is due to fractional contraction. The problem of the transmission mechanism, in which the impulse in one axon with many terminals activates some nerve ends in muscle while others remain relatively inactive, is still unsolved. It is a fact that one axon may divide and terminate in two nerve ends in two neighboring muscle fibers. One nerve end may be expanded while the other is retracted. The nature of this alternating, shunting mechanism in the conduction of the nerve impulse resulting in differential rest and motion of the fibers in a single muscle is still unknown.

Specific structural changes produced by the strong electric current in muscle fibers were best studied in the winter frog, or in the summer frog after it had been in the refrigerator at 6° to 8° C. for 48 hours (Figs. 68 and 69). The nuclei in the muscle fibers of either naturally or artificially hibernating frogs became centrally located in the muscle fiber. When contracture was produced by electricity the myoplasm in the single muscle fiber was arranged in a series of alternating condensed nodes and rarefied internodes. The nuclei were compressed in the nodes of condensation and stretched in the internodes of rarefaction. These condensed nodes and rarefied internodes were assumed to be the two physical components of a train of longitudinal waves of compression that accompany the variable capillary chemical reactions in the microcapillary muscle fiber.[13] The periodicity of the structure of both the normal and abnormal muscle fibers was dependent upon both the physical and chemical factors associated during changes in the capillary chemistry. These histologic findings confirm the physiologic observations on muscle contracture reviewed by Gasser [29] and those of Tower [30] on the reaction of muscle to denervation.

Summary

Evidence is presented that functional ameboidism determines the pleomorphism of both the normal and abnormal motor nerve plates in intercostal muscle. The respiratory rate in rats subjected to carbon dioxide was accelerated from 30 to 200 per minute with labored breathing and hyperpnea. Direct evidence is presented, based on the effect of hyperpnea by carbon dioxide, that the motor nerve plate physically vibrates during functional activity. It expands when the muscle fiber contracts and constricts or retracts when the muscle fiber relaxes.

The retracted motor nerve plates with wide, short projections are related to coarse, widely spaced cross striations, whereas expanded nerve plates with thin, elongated and moniliform processes are longer and separated by wider meshes than the retracted ones and are related to fine, closely spaced cross striations (Fig. 17). Over 60 per cent of the expanded motor nerve plates in the intercostal muscle in which the rate of transmission of the nervous impulses was accelerated by carbon dioxide became twice the size of the relatively normal plates. Their processes were longer and separated by wider meshes than those of the normal controls. This morphologic expansion and extension of the terminal arborization in the nerve plate by ameboid motion under the stimulus of the demand of increased functional activity evidently favors the transmission of the nerve impulse to the receptive muscle substance by increase of the surface area of the plate.

The differences in the histologic structure of motor nerve plates and muscle cross striations are arranged in a graded series and evidently are determined by the active and inactive fibers in the fractional contraction of the same muscle. These structural changes favor the chemical theory of impulse transmission. Ameboid motion explains the pleomorphism of the motor nerve plates fixed in the closely related active and inactive muscle fibers of a muscle.

Strong stimulation by carbon dioxide and electricity produces a pathologic explosion of the ameboid structure of the motor nerve plates with turbulence of the muscle cross striations. The pattern of the cross striations in intercostal muscle is as variable as that of the internal energy associated with the capillary chemistry confined in the microcapillary muscle fibers. There is no constant unit of structure, such as the so-called sarcomere, in the intercostal muscle fiber. Therefore, (1) functional ameboid motion determines the pleomorphic structure of the motor nerve plates, and (2) changeable capillary chemistry determines the pleomorphic nature of the variable periodic structure in striped muscle.

NOTE: I wish to express gratitude to Leo Massopust, Director of the Department of Art and Photography, for aid with the photomicrographs; to G. Kasten Tallmadge, Assistant Professor of Anatomy, for reading the manuscript, and to Stephen Chess and Eugene Haushalter for technical aid in the teasing of muscle and nerve plates.

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DESCRIPTION OF PLATES

All of the untouched photomicrographs, Plates 35 to 51, are from the teased right intercostal muscle fibers of the white rat, consisting of: 1, relatively normal muscle and nerve plates; 2, those subjected to carbon dioxide stimulation; and 3, those electrically stimulated through the spinal cord by the ordinary alternating house current, 120 volts, 1 ampere, for 1 minute.

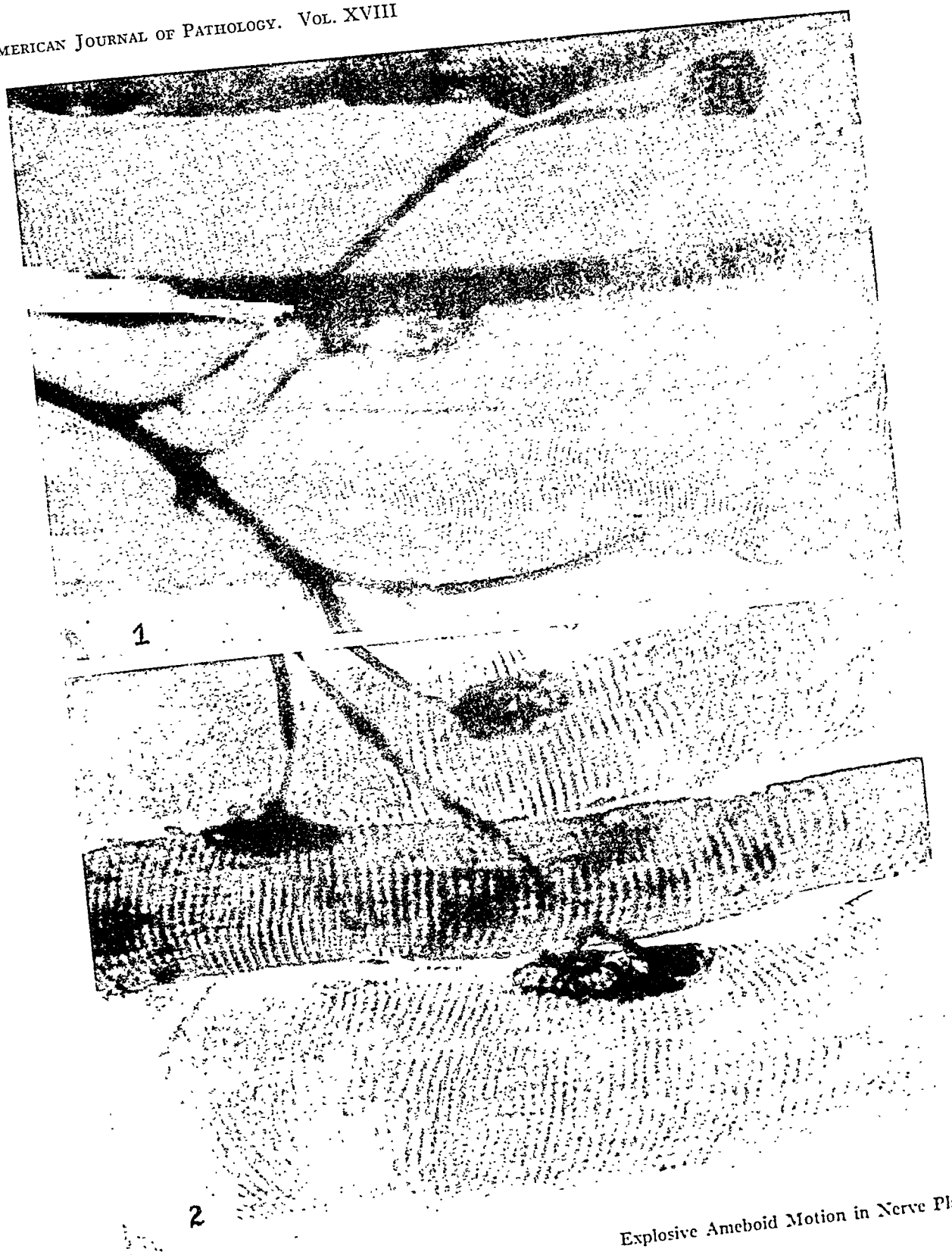
Plates 35 to 38 are photographs of the teased right intercostal muscles of rat no. 25. It is important to note that Plates 39 to 51 are photographs of the teased right intercostal muscles and contain pleomorphic motor nerve endings from the same animal, rat no. 57. All were prepared by the gold chloride technic. All figures in Plates 36 to 51 are magnified 750 ×. In Plate 35, Figure 1 is magnified 300 ×; Figure 2 is magnified 750 ×.

PLATE 35

FIG. 1. Sprays of medullated nerve fibers and motor end-plates in normal teased intercostal muscle fibers, white rat no. 25. The darkly stained relaxed muscle fibers have coarse, widely spaced cross striations, whereas the lightly stained contracted muscle fibers have fine, closely spaced striae. There are many transitional stages between these two extremes. The upper pair of motor nerve plates are derivatives of a single axon; one branch terminates in a retracted motor plate in a relaxed muscle fiber, the other terminates in an expanded nerve plate in a contracted muscle fiber. The middle pair of nerve plates is composed of one retracted and one expanded plate, derived from a single axon. These normal teased intercostal muscles were obtained before the rat was subjected to CO_2.

FIG. 2. Normal teased intercostal muscle fibers, white rat no. 25, gold chloride. The central darkly stained, relaxed muscle fiber has coarse, widely spaced cross striations, whereas the expanded nerve plates in the two contracted fibers have variable degrees of fineness and spacing of the related cross striations. The expanded nerve plates in the active muscle fibers have variable degrees of ameboid extensions of the terminal arborization of the axon. There appears to be a centrifugal extension of the terminal arborizations of the axon of the nerve plate, with increase of the surface area during muscle contraction; and a centripetal retraction of processes, with decrease of the surface area, during relaxation of the muscle fiber. Fractional contraction, or some fibers active with others at rest, appears to be the cause of these differences in structure. The active motor nerve plates have a greater number of dichotomous terminal divisions than the inactive ones. There are 15 dark striations related to the retracted motor plate on the middle muscle fiber and 24 related to the expanded nerve plate on the lower muscle fiber.

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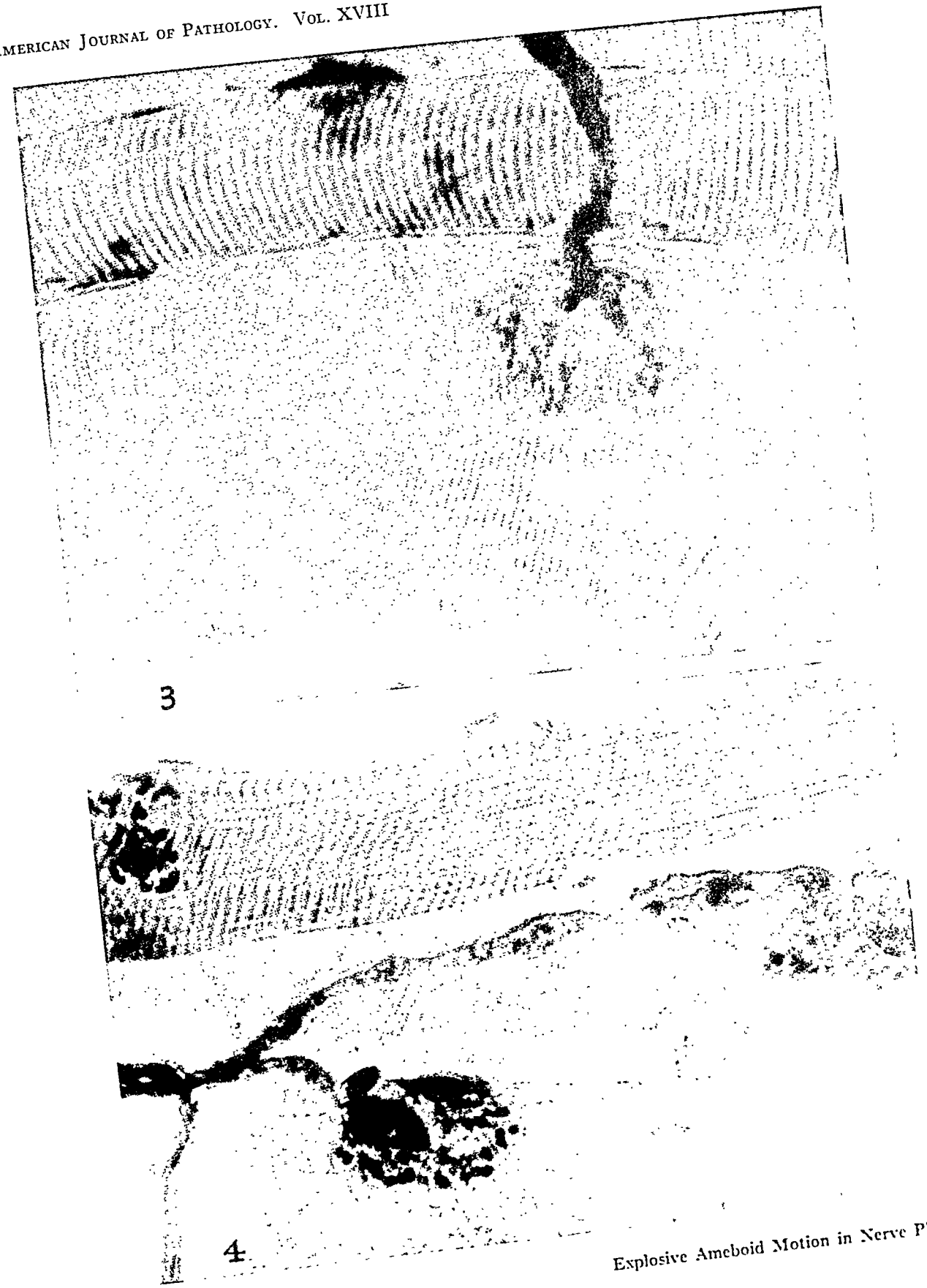
Explosive Ameboid Motion in Nerve Plates

Carey

PLATE 36

FIGS. 3 and 4. Normal coarsely and finely striped muscle fibers. There is an irregular radiation pattern of the fine, closely spaced cross striations in relation to the expanded ending in the lower muscle fiber (Fig. 3). These cross striations become coarser and more widely spaced with increase of the distance from the nerve plate toward the left of this fiber. Variations in the end-plates and muscle striae are also observed in Figure 4. The diameter is not a reliable basis for classification of muscle fibers. Some wide fibers have coarse striations and some narrow fibers contain fine striations. This is in part dependent upon whether the muscle fiber is fixed in a state of isotonic or isometric contraction. In some fibers there is a simple alternation of dark Q and light J bands. The Q and J bands vary in width. In some plates there is a doubling of the Q band and in others the pattern of the so-called constant sarcomere structure of Z, J, Q, J, Z. This pattern, however, is highly inconstant. There is, therefore, no constant number of so-called sarcomeres in this striped muscle. The active motor nerve plates have a greater number of axonal ramifications than the inactive ones. There are 14 dark striations related to the retracted motor plate on the upper muscle fiber and 29 dark striations related to the expanded plate on the lower muscle fiber (Fig. 3).

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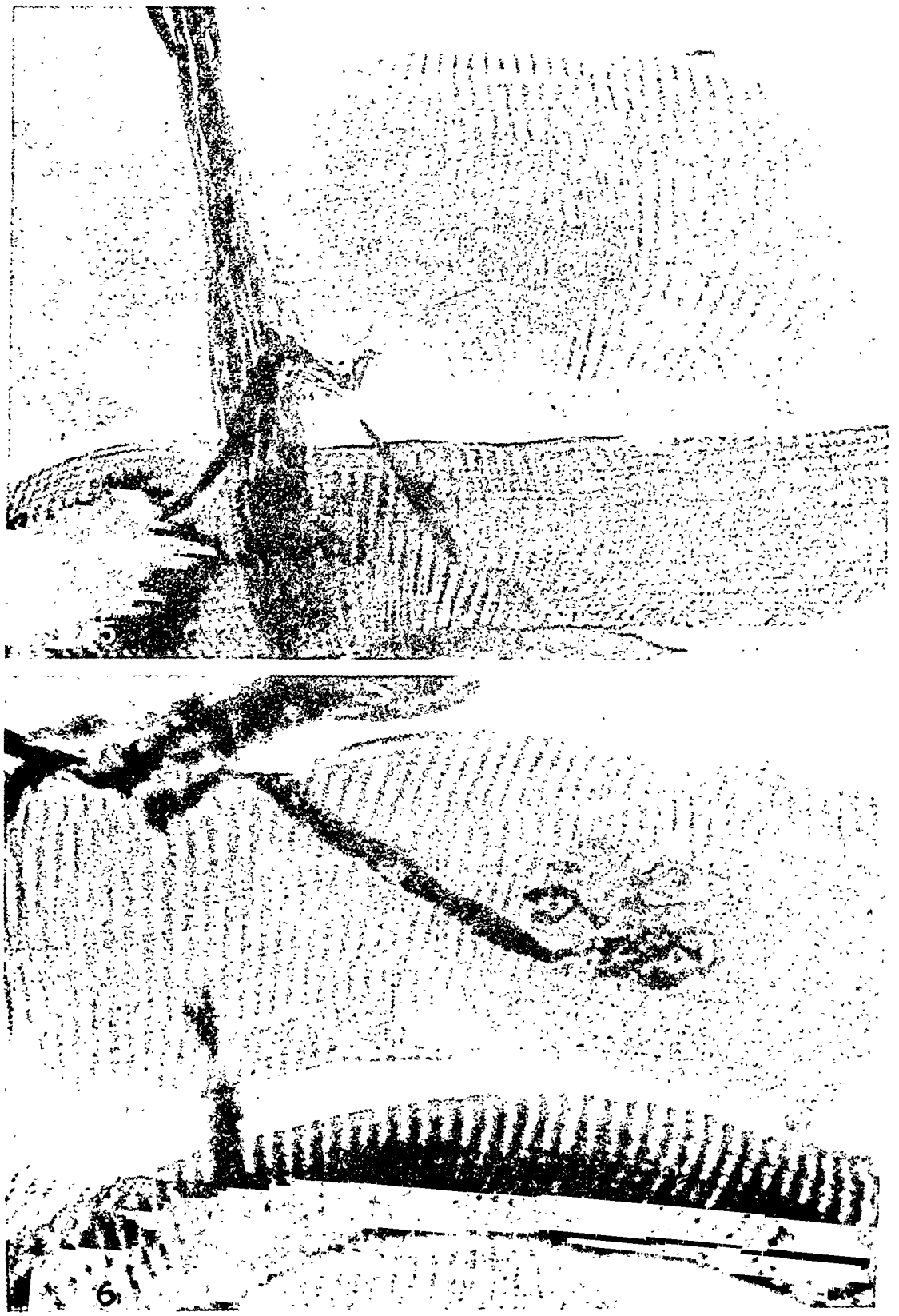


Explosive Ameboid Motion in Nerve Plates

Carey

PLATE 37

FIGS. 5 and 6. Normal relaxed dark and contracted light muscle fibers containing retracted and expanded motor end-plates, respectively. There is a granular obliteration of the cross striations to the left of the upper light muscle fiber in Figure 5 and to the right of the upper light muscle fiber in Figure 6. The wide dark bands with relatively narrow light bands are clearly seen in the narrow, resting and dark lower muscle fiber (Fig. 6). With progressive increase in intensity of contraction the dark Q band narrows, the light J band broadens, the Z band appears and a duplex Q band occurs. The pseudopodia of the nerve plate in the upper fiber (Fig. 6) are dichotomously branched into tuning-fork-like processes. The nuclei of the granular sole plate of Kühne are found within the crotch of the divisions of the axon. The active motor nerve plates have a greater number of axonal branches and a greater number of related cross striations than the inactive nerve plates.



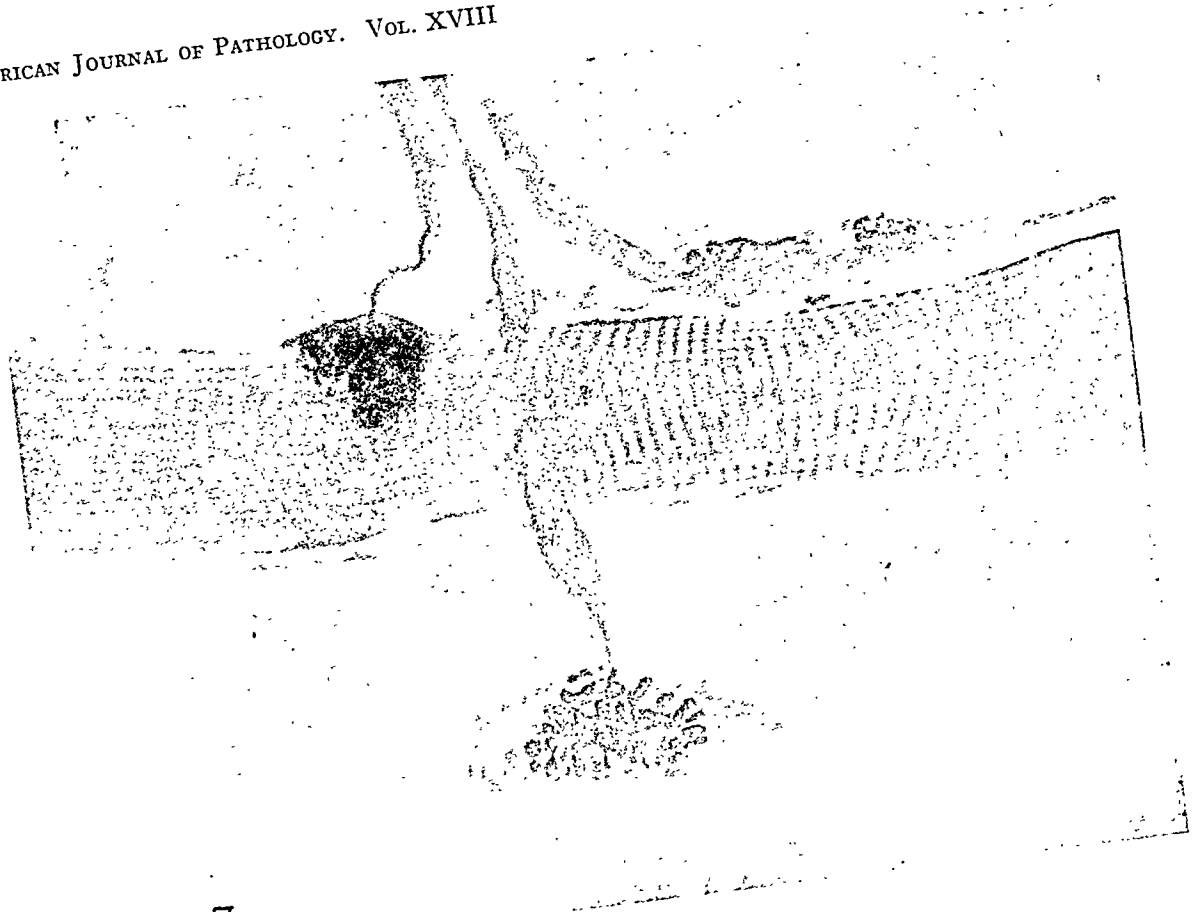
Carey Explosive Ameboid Motion in Nerve Plates

PLATE 38

FIG. 7. The teased right intercostal muscles and motor nerve ends (Figs. 7 and 8) are from the same animal. One normal coarsely striated muscle fiber lies between two finely striated ones. The retracted nerve plate on the coarsely striped muscle fiber has a dark granular sole plate of Kühne. This sole plate has undergone progressive dissolution in the two expanded nerve plates. The oval clear areas at the left and right poles of the lower nerve plate are occupied by nuclei of the granular sole plate of Kühne.

FIG. 8. Expanded motor nerve plate and muscle fiber stimulated for 1 minute with CO_2. This expanded nerve plate appears to be formed during superfunctional activity by the streaming out of the protoplasm into the sarcoplasm centrifugally from the proximal axonic axis. There is dichotomous branching and anastomotic reticulation of the branchings of the axon in the nerve plate. In some places there are terminal enlargements of the ramifications of the axon in the nerve plate. This enlargement of the nerve plate during increased functional activity is objective evidence of the transference of substance from the nerve to the muscle. The dark Q bands are double and form an irregular radiation pattern around the nerve end. To the left of the nerve end there is an obliteration of the striated pattern with the replacement of the cross striations by granules. The vascular loop surrounding the motor nerve plate, called *confluens capillorum* by Wilkinson,[20] is clearly evident. The vessels that form the loop become more dilated than normally upon strong stimulation by either CO_2 or electricity. The motor nerve plate stimulated by CO_2 has a greater number of axonal arborizations than the inactive one. This stimulated plate appears to possess the capacity of neurocladism in the terminal turgescent bulb. This is the resultant of divisional ameboidism of the terminal nerve bulb upon strong stimulation. The resistance presented by the sole-plate nuclei of Kühne evidently favors the division of the protoplasmic streaming of the terminal pseudopodia of the axonic bulb. There are 11 dark striations related to the retracted nerve plate on the middle muscle fiber (Fig. 7) and 35 dark striations related to the expanded nerve plate (Fig. 8).

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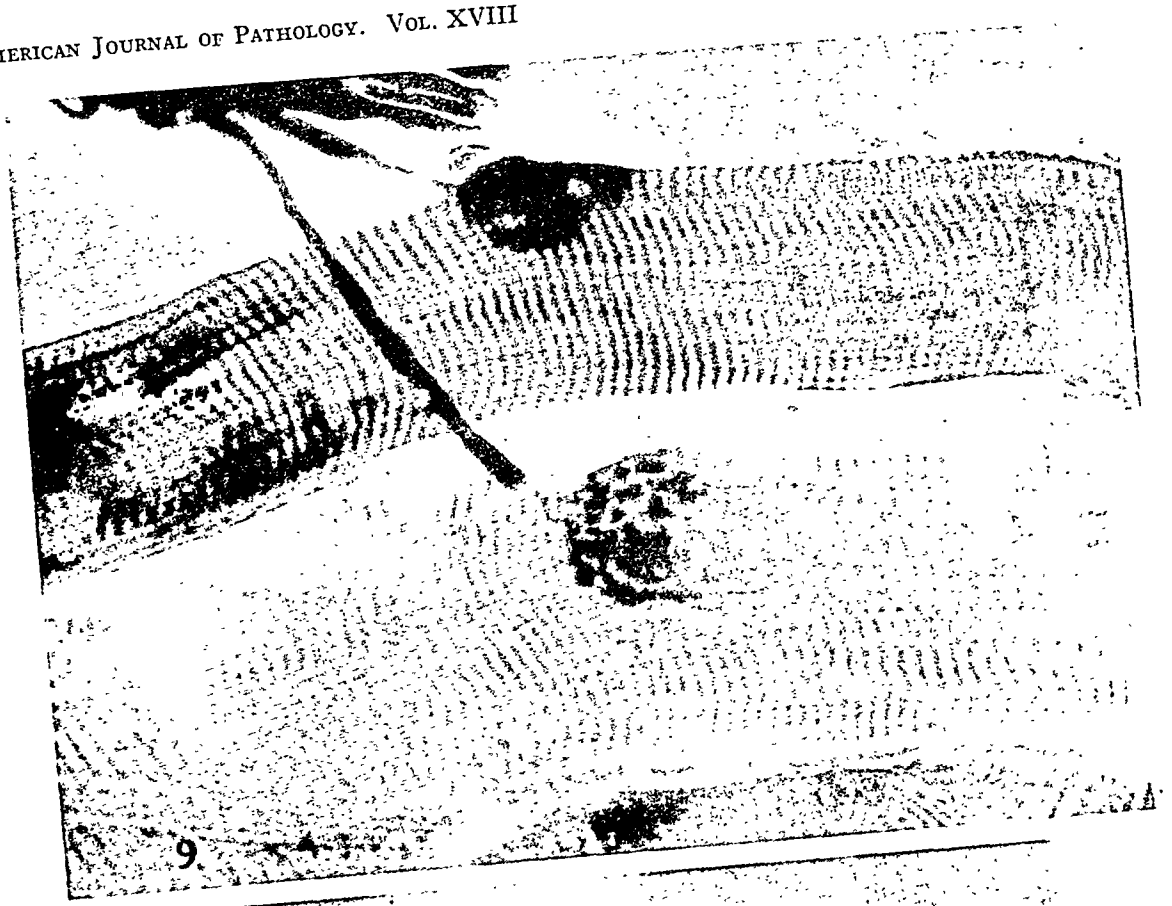
Explosive Ameboid Motion in Nerve Plates

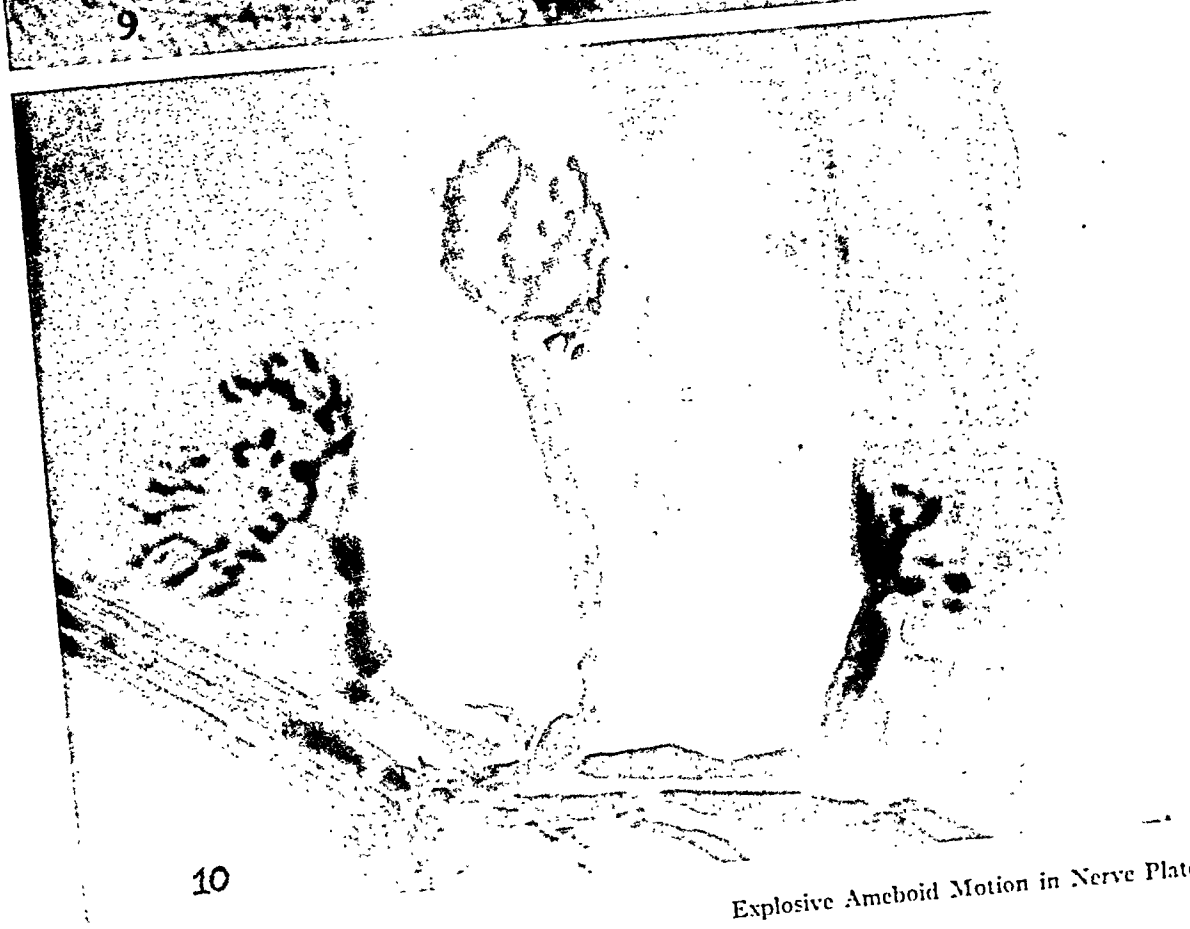
PLATE 39

FIG. 9. Normal dark and light intercostal muscle fibers. The narrow dark and relaxed muscle fiber has coarse darkly stained cross striations and a retracted motor nerve plate surrounded by Kühne's granular sole plate. The light muscle fiber has fine closely spaced cross striations close to the slightly expanded motor nerve plate. The granular sole plate of Kühne is undergoing dissolution. The cross striations vary in fineness and spacing and in the relative proportions of space occupied by the dark and light bands.

FIG. 10. Intercostal muscle fibers, stimulated for 1 minute with CO_2. With progressive increase of ameboid expansion of the motor nerve plates there is a corresponding increase in number and fineness of closely spaced cross striations. The strongly contracted muscle fibers have large motor nerve plates with two or more moniliform projections separated by wide spaces. With progressive increase in size of the motor nerve plate and increase of fineness of the dark and light transverse gratings the contracted muscle fiber takes a lighter strain. There are many transitional stages and gradations between the coarsely and finely striated muscle fiber. There is a substantial ameboid protrusion of the processes of the nerve plate in various directions in the muscle substance which increases the surface area of the nerve plate.

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Explosive Ameboid Motion in Nerve Plates

PLATE 40

FIGS. 11, 12 and 13. Progressive expansion of the motor nerve plates and change in periodic structure of the cross striations in the intercostal muscle stimulated by CO_2 for 1 minute. There appears to be a centrifugal flow of substance or protrusion of the axonic terminals of the nerve plate into the muscle substance from the retracted state (Fig. 11) to that of the variable degrees of expansion (Figs. 12 and 13). Ameboid motion is the changeable play in the centrifugal expansion and centripetal retraction of the axonic pseudopodia. The coarse muscle striae are related to the retracted plate and the fine striae to the expanded ones. There is a richly branched pseudopodial network in the lower expanded nerve plate (Fig. 13). The clear ovoid areas within and at the periphery of the nerve plates are occupied by the nuclei of the sole plate of Kühne. The irregular radiation pattern of the striae defy description in words. There is a massive transference of nerve substance into the axonal ramifications in the expanded motor nerve plates stimulated by CO_2.

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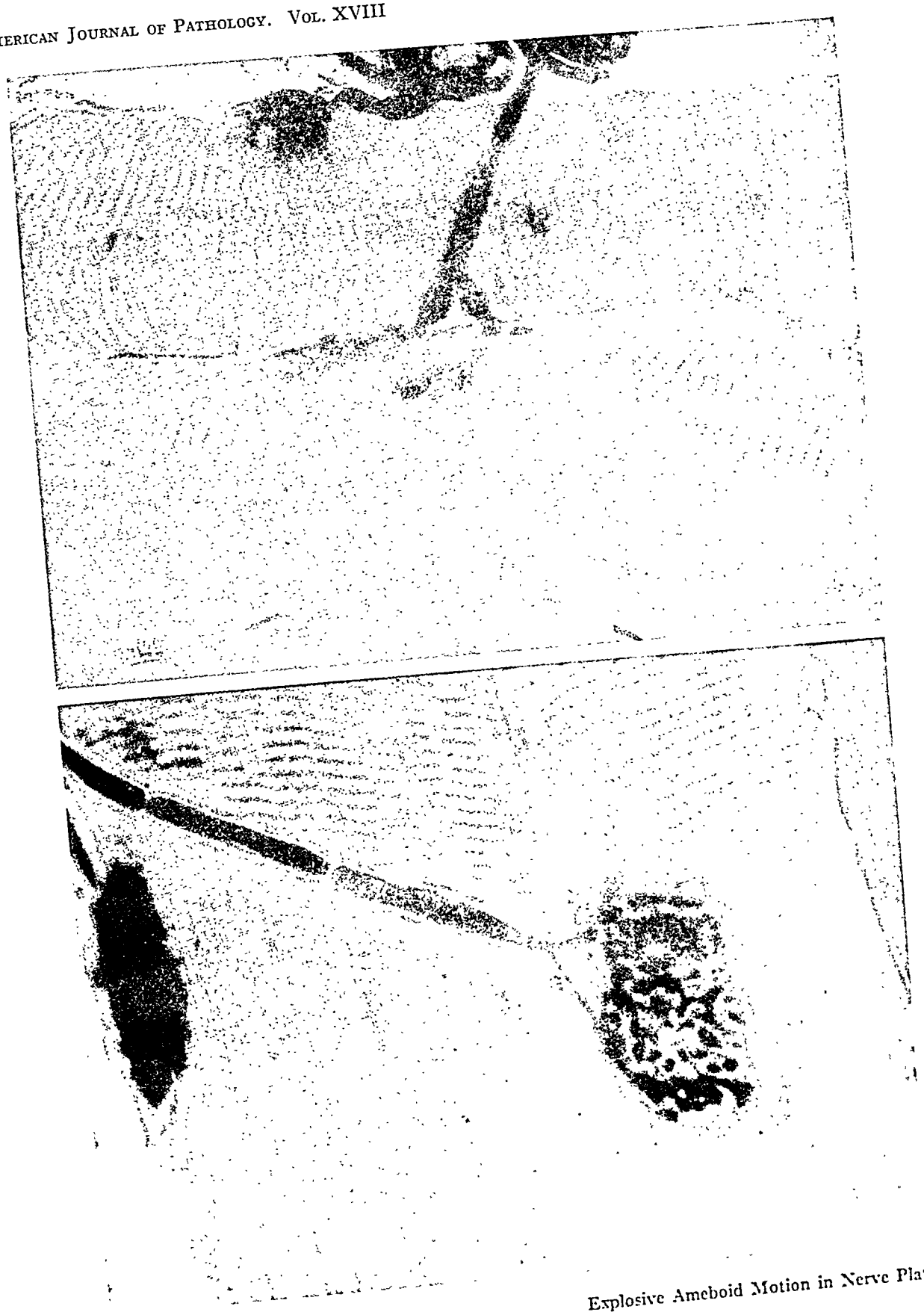


Explosive Ameboid Motion in Nerve Plates

PLATE 41

FIGS. 14 and 15. Intercostal muscle fibers and motor end-plate stimulated to hyperpnea with CO_2 for 1 minute. There is a dichotomous division of the axon before it terminates on the nerve plate of the lower muscle fiber (Fig. 14). There is an obliteration of the muscle striae below this plate, and a biconvex radiation of the muscle striae toward the left and right in the muscle fiber. These changes are observed in nerve and muscle fixed *in situ* under normal tension of the terminal skeletal attachments. This pattern in the muscle is not one due to abnormal optical effects in photography, to crushing in teasing nor to the pressure of the coverslip. This localized obliteration of the striations underlying the double motor nerve plate is observed at all levels of the focus. This motor plate is composed of two discrete components contributed by the dichotomous division of the single axon. There is a gradient in the fineness and spacing of the cross striations in the different phases of the functional activity of the muscle fiber in fractional contracture. There is a polar connection of the medullated axon with the motor nerve plate (Fig. 15). The widened dark axon is separated from the fine dark linear neurilemma by the clear medullary sheath which fails to take the gold deposit. The neurilemma is continuous with the sarcolemma. Immediately underneath, to the right of and below each nerve plate, the fine dark striations are irregular and closely spaced. Above the nerve plates the dark cross striations are coarse and widely spaced. There is a faint Z band between the coarse Q bands, bounded on each side by half J bands. This Z band is highly inconstant in the highest magnifications of the muscle fiber. When the motor nerve plate expands at one pole more than at the opposite one there is frequently a differential pattern to the related cross striations in a single muscle fiber. At the lower expanded pole (Fig. 15) the striae are fine and irregular. At the upper relatively non-expanded pole the striae are coarse and relatively regularly spaced. The oblong clear areas at the periphery of the nerve plates, in the granular sole plates of Kühne, are occupied by nuclei. There is a massive conduction of nerve substance into the axonal ramifications in the expanded motor nerve plates, stimulated by CO_2.

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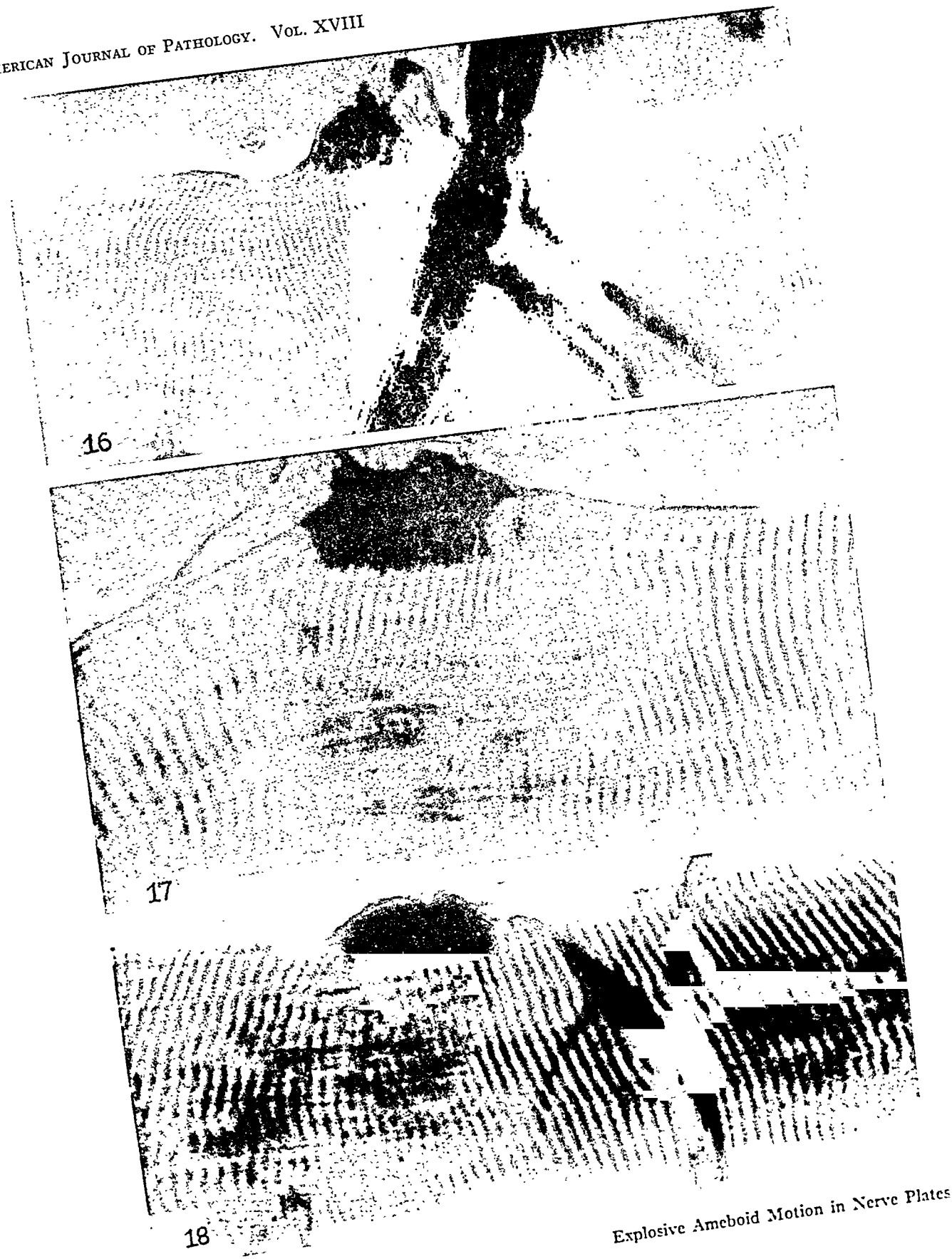
Explosive Ameboid Motion in Nerve Plates

PLATE 42

FIG. 16. The hypolemmal motor nerve plate in profile view is related to a pattern of muscle striae called the typical *Noniusfelder* of Heidenhain. The variable radiation and rhythmic replacement of the cross striations related to the functional activity of the motor nerve plate in the muscle fiber may explain the differential morphology of the cross striations of muscle (Figs. 16, 17 and 18). This is from the third left intercostal muscle, 1 minute after exposure in an atmosphere of CO_2.

FIGS. 17 and 18. Irregular granular zones in the muscle fibers are related to motor nerve plates. The coarse striations at the ends of the muscle fibers are replaced by those related to an activity which appears to wipe out the striations underlying the nerve plates. This obliteration of the striations appears to occur at the beginning of certain degrees of intensity of the propagated disturbance that radiates through the muscle fibers from the motor nerve plate. These are radiation areas of contracture in muscle, beginning at the nerve plate. This subsequently results in a graded series of the spacing and width of the dark cross striations when fixed at the initial phases of expansion of the motor nerve plate in striated muscle. The pattern of the cross striations in muscle underlying the nerve plate is highly inconstant.

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Explosive Ameboid Motion in Nerve Plates

PLATE 43

FIG. 19. Two teased right intercostal muscle fibers, stimulated with CO_2. The nerve plates are in profile and the related muscle striae are fine and closely spaced to the right, and coarse and widely spaced to the left of the plate. This differential structure of the muscle striae is related to differential expansion of the nerve plate.

FIGS. 20 and 21. Right intercostal muscle fibers with periodic groups of fine striations alternating with coarse striae. To the right of the upper muscle fiber (Fig. 20) each S-shaped, coarse stria divides below into two fine striae. These nodes of contracture composed of fine striations are radiated in groups from the irregularly active motor nerve plates.

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Explosive Ameboid Motion in Nerve Plates

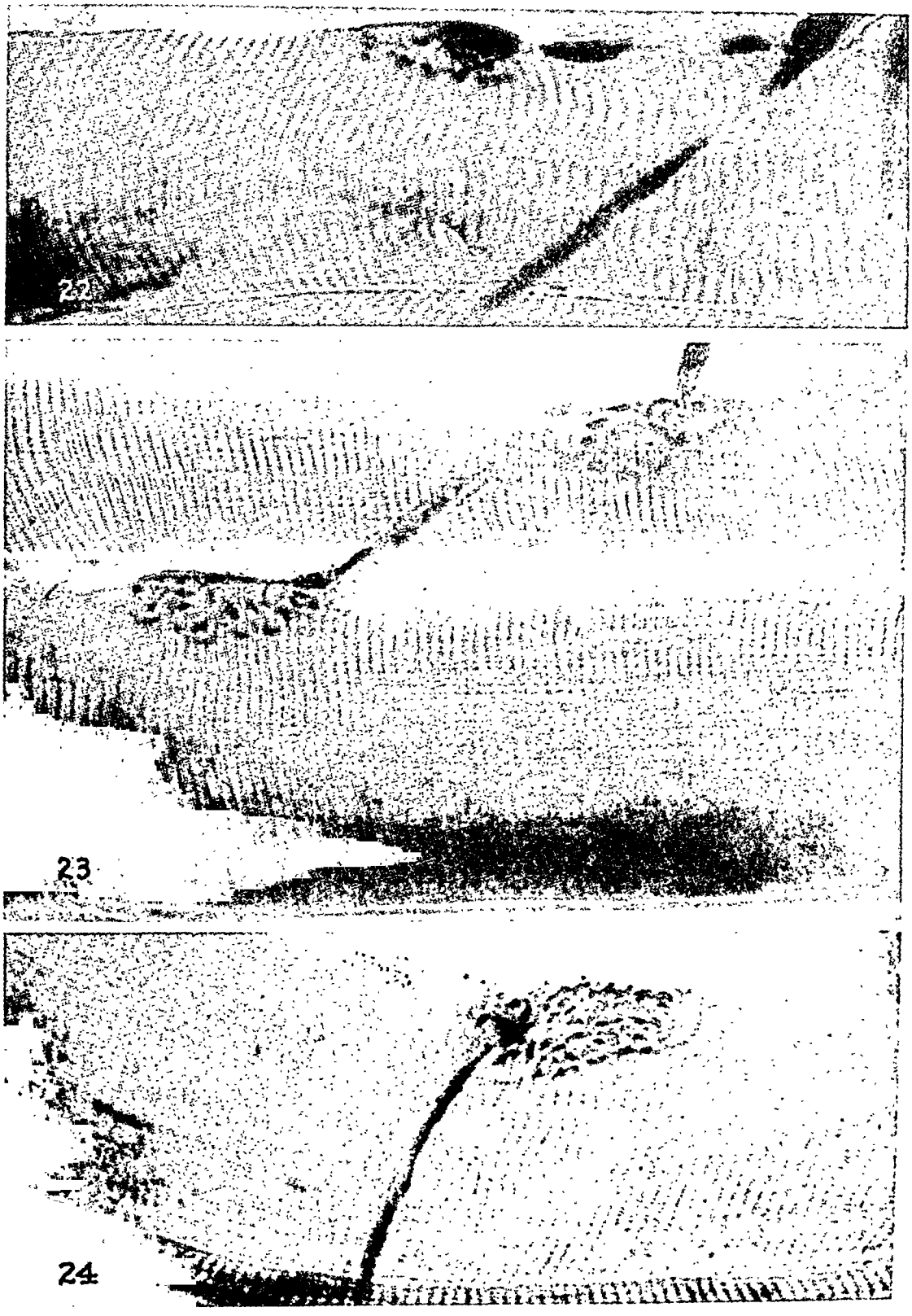
Carey

PLATE 44

FIG. 22. Intercostal muscle fiber; hyperpnea by stimulation for 1 minute with CO_2. The retracted motor nerve plate, surrounded by the granular sole plate of Kühne, has fine, closely spaced cross striations related toward the left, and wide, closely spaced striae toward the right of the muscle fiber.

FIG. 23. The upper motor nerve plate has a circular outline, whereas the lower one is more expanded. The cross striations have in some places a doubling of the Q band, and in others a Z, J, Q, J, Z pattern. In still other regions the striae have a spiral pattern.

FIG. 24. Toward the right of the muscle fiber the dark Q bands alternate with the light J bands. Toward the left there is an irregular criss-cross pattern. There are four branches of the single axon in the motor nerve plate. Toward the right the ovoid space is occupied by a nucleus of the granular sole plate of Kühne. The muscle fibers of this plate illustrate the varying coarseness and fineness of the cross striations and related degrees of retraction and expansion of the motor nerve plates, respectively.



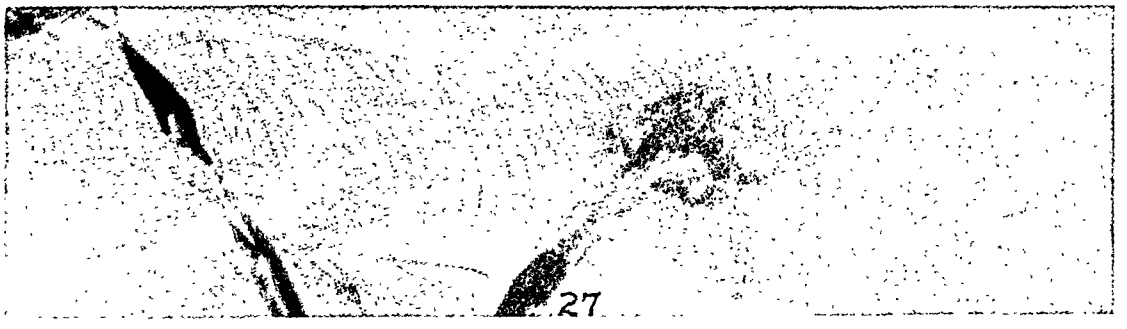
Carey Explosive Ameboid Motion in Nerve Plates

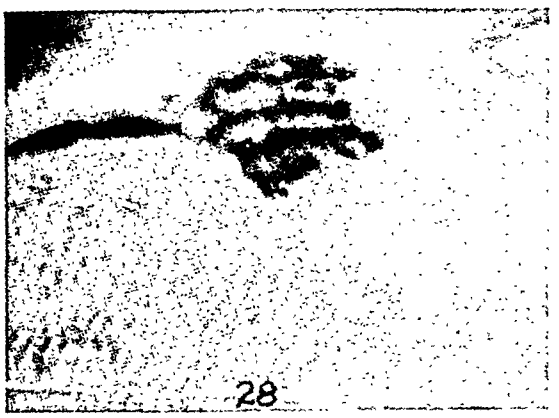
PLATE 45

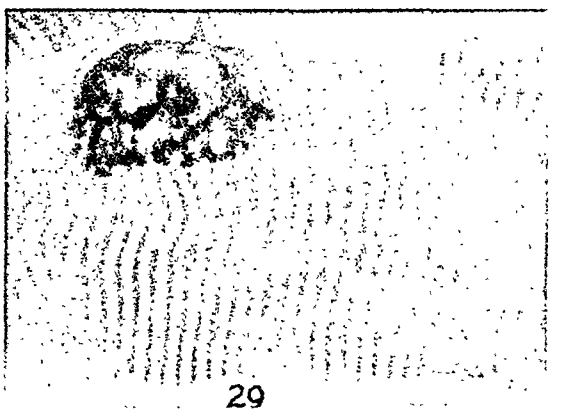
FIGS. 25 to 31. Pleomorphism by ameboid changes of axonic branches in motor nerve plates in teased intercostal muscle fibers; hyperpnea by CO_2 stimulation for 1 minute. The retracted and slightly expanded nerve plates are surrounded by the dark granular sole plate of Kühne (Figs. 25, 26 and 27). This granular sole plate disappears in the expanded plates of the highly active muscle fibers. There are 11 dark cross striations related to the retracted nerve plate in the relaxed muscle fiber (Fig. 25). There are 32 dark cross striations related to the expanded motor nerve plate in the active muscle fiber (Fig. 31). This differential numerical relationship of the muscle striae related to the retracted and expanded nerve plates has a definite relationship to physiologic and pathologic functions at the myoneural junction. In the superactive muscle fibers (Figs. 30 and 31) it is objectively evident that there is a substantial increase in size of the motor nerve plates. The clear ovoid areas, both within the nerve plate and in the muscle substance, are occupied by nuclei of the granular sole plate of Kühne. The vascular loop surrounding the motor nerve plate, called *confluens capillorum* by Wilkinson, is clearly evident. There is a massive conduction of nerve substance into the axonal ramifications in the expanded motor nerve plates, stimulated by CO_2. The ultra-termination of the axonic pseudopodia of the motor nerve end with muscle is highly variable. There is no constant Z or Krause membrane, or other periodic structure, or periterminal network.

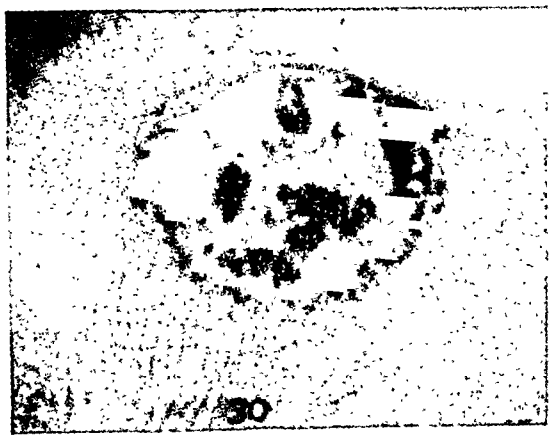

25


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27


28


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31

Plate 46

Figs. 32 to 35. Pleomorphism by ameboid changes of axonic branches in motor nerve plates in teased muscle fibers, hyperpnea by CO_2 stimulation for 1 minute. The retracted plate (Fig. 32) has the clear ovoid places occupied by the nuclei of the granular sole plate of Kühne close to the nerve plate. With progressive explosive expansion of the plates by ameboid motion and sudden chemical changes these clear oval areas are projected into the muscle substance and at some distance from the nerve plate (Figs. 33, 34 and 35). There is a replacement of the clearness of periodic structures in the muscle by the irregular placement of granules. The ovoid clear areas in the muscle substance occupied by nuclei are surrounded by a dark granular radiation. In some places these clear oval areas may resemble vacuoles in both the nerve plate and in the muscle. Superactivity evidently favors neurocladism, or dichotomous division of the branches of the axon in the nerve plate (Figs. 33, 34 and 35). The obstacles composed of nuclei of the granular sole plate of Kühne found in the crotch of the dividing terminal axon may condition the morphology of the expanding motor nerve plate during superfunctional ameboidism. There is a massive transmission of nerve substance into the axonal ramifications in the expanded motor nerve plates when stimulated by CO_2. There is divisional ameboidism (Fig. 34) of the terminal axon, the projections of which grow out by superfunctional stimulation among and beyond the nuclei of the granular sole plate of Kühne. These projections normally do not extend beyond the granular material of the sole plate of Kühne. The strong chemical, CO_2, acts as a neoformative stimulus to the production of the axonic branches of the motor nerve plate. There is a varicose, or moniliform, pattern to the axonic ramifications. When the axon is strongly stimulated and its terminals meet the resistance of nuclei and other protoplasmic obstacles there results a precipitous and prolific projection of ramifications (Fig. 34). The dichotomous divisions extend between the nuclei of the dark granular sole plate of Kühne. The protoplasmic movement is the result of capillary chemical change in concentration or composition, or both, of the axonic protoplasm with a consequent modification of the internal pressure which extends along the lines of least resistance.

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Explosive Ameboid Motion in Nerve Plates

PLATE 47

FIGS. 36 to 39. Pleomorphism of retracted and expanded motor nerve plates in right intercostal muscle, after 1 minute of hyperpnea by CO_2 stimulation. The relatively slightly active, inactive and retracted motor end-plates have short, wide, coarse fronds (Figs. 36 and 37). The superactive and expanded motor nerve plates have long, narrow pseudopodia, or terminal branches, of the axon in the nerve plate. Concomitant with the explosive expansion of the nerve plate (Figs. 33, 39, 50, 58 and 63) there appears to be an explosive replacement of the muscle striae by granules. At great distances to the left and right of the nerve plate and in the granulation of the muscle substance, there are found clear areas which contain the unstained nuclei. These nuclei have been projected into the muscle substance, and away from their close peripheral position to the nerve plate. These nuclei in the muscle substance have the same morphology, continuity and relationship to the surrounding radiation of granules as those that still maintain the close position in the intact sole plate of the motor axonic nerve plate. These explosive patterns in muscle and nerve are not artefacts due to compression and teasing of the muscle fibers. They are found likewise in muscle fixed intact in the animal and then serially sectioned. The best evidence is obtained, however, when the continuity of the motor nerve plate and muscle fiber is preserved by gently teasing and preserving them together by the gold chloride method. There is a massive transportation of nerve substance into the axonal ramifications in the expanded motor nerve plates, stimulated by CO_2.

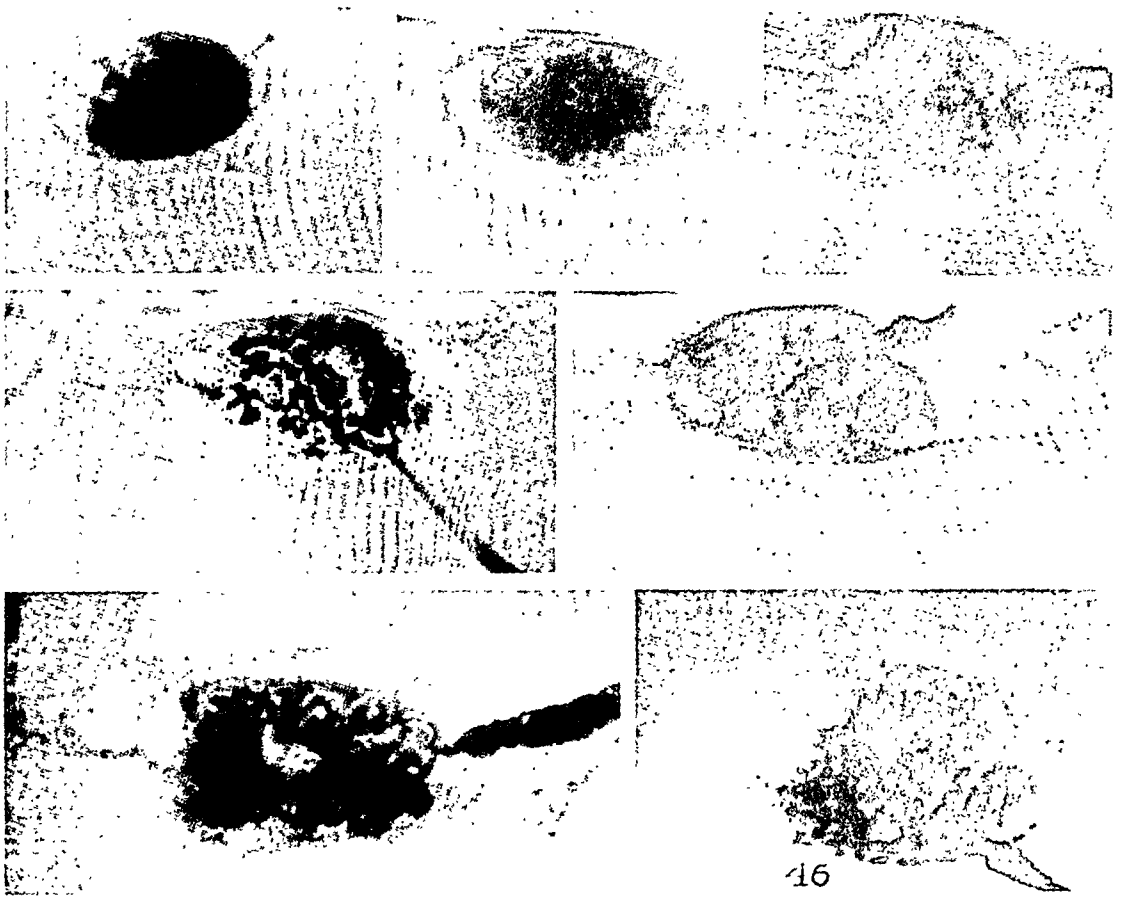


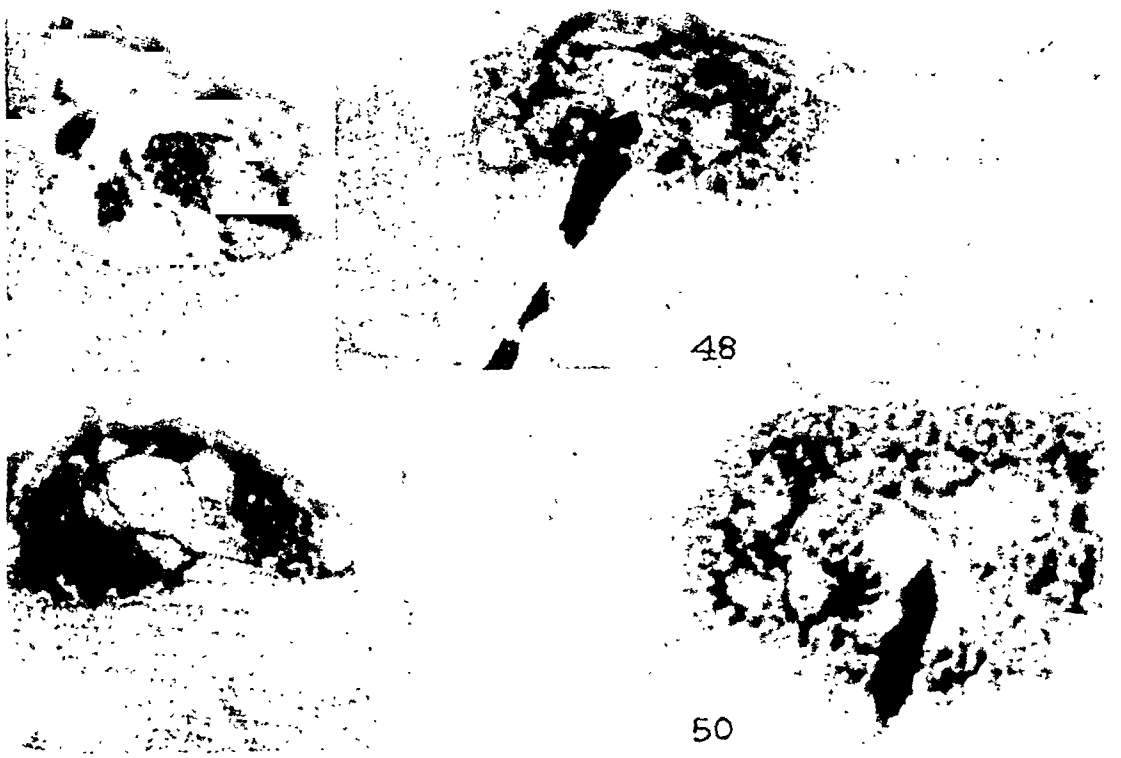
Explosive Ameboid Motion in Nerve Plates

Carey

PLATE 48

FIGS. 40 to 50. Pleomorphism of the motor nerve plates, by superfunctional ameboidism of the axonic terminals, in teased right intercostal muscle fibers: hyperpnea due to CO_2 stimulation for 1 minute. The clear ovoid nucleated areas, which appear like vacuoles, are both closely related to the periphery of the nerve plates, within the granular sole plate of Kühne, and displaced at some distance in the muscle substance by some explosive force related to the sudden expansion of the motor nerve plate. Note that in the direction in which the nuclei are projected from the periphery of the nerve plate into the muscle substance there is a streamlike effect in the sarcoplasm and a replacement of the regular pattern of cross striations by an irregular arrangement of granules (Figs. 43, 44, 45, 46, 48 and 50). There is a direct continuity by a streamline of granules between the clear ovoid and fusiform areas in the muscle substance and the granules of the sole plate of Kühne surrounding the axonic branches of the motor nerve plate (Figs. 44, 45, 48 and 50). There is a massive conduction of nerve substance into the axonal ramifications in the expanded motor nerve plates, stimulated by CO_2. The motor nerve plates are morphologically lawless and become explicable only on the physiologic and pathologic basis of the dynamics of functional ameboidism. The retraction of the pseudopods of axonic arborization toward the base of the stem forms a wide terminal bulb or cone (Fig. 40). This terminal bulb has wide, short lobes. The pleomorphism and acute hypertrophy of the motor end-plates are produced by ameboid expansions and extracentral ramifications and reticulations of the numerous axonic projections under the strong stimulus of CO_2. The nerve end-plate is a plastic mass undergoing constant change in chemical composition and concentration, with resultant morphologic changes from a small or large bud (Fig. 40) to a large bouquet with extensive ramifications (Fig. 50). The tuberous arborizations of the sprouting axon become progressively thinner with varicosities at the bifurcations and terminals of the processes. Accumulation of protoplasm or intumescence occurs at the end of each branch of the axon. The branches take different configurations and terminate in end rings, nets, spherules, or as finger-like terminals.



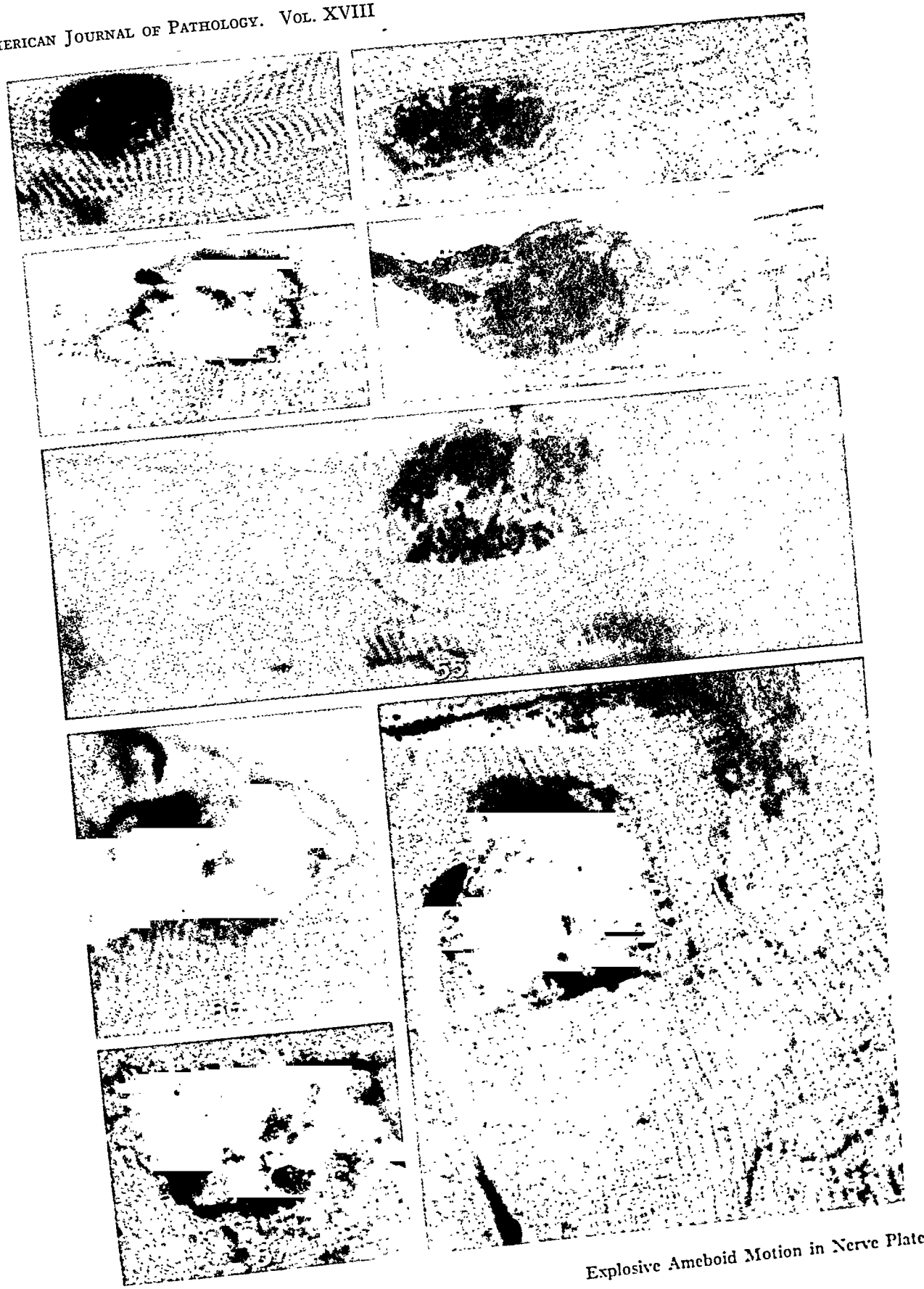


Carey Explosive Ameboid Mo[illegible] Neur[illegible]

PLATE 49

FIGS. 51 to 58. Pleomorphism of the motor nerve plates produced by superfunctional ameboidism of the axonic terminals in teased right intercostal muscle fibers; hyperpnea due to CO_2 stimulation. The retracted motor nerve plate (Fig. 51) has 13 dark striations in relation with its granular sole plate of Kühne. The partially expanded nerve plate (Fig. 55) has 45 dark cross striations in relation with its remains of the granular sole plate and the axonic motor nerve plate. The single nucleated oval clear area is in a direct stream-like continuity with the nucleated ovoid clear area in the granular sole plate of Kühne (Fig. 52). This stream-line of granules and of irregular granulation of the sarcoplasm is clearly evident (Figs. 47, 53, 54 and 58). The vascular ring around the motor nerve plate, *confluens vasculorum* of Wilkinson, is evident. There are fine oval and fusiform nucleated clear areas in the sarcoplasm scattered at a distance from the motor nerve plate. The irregular scattering of the oval clear areas is likewise shown (Fig. 58). These oval, clear areas, containing nuclei, are scattered out in the muscle substance. They occur in great frequency in practically all muscle fibers, the motor end-plates of which have been greatly expanded by CO_2 stimulation. There is a massive transmission of nerve substance into the axonal divisions in the expanded motor nerve plates, stimulated by CO_2.

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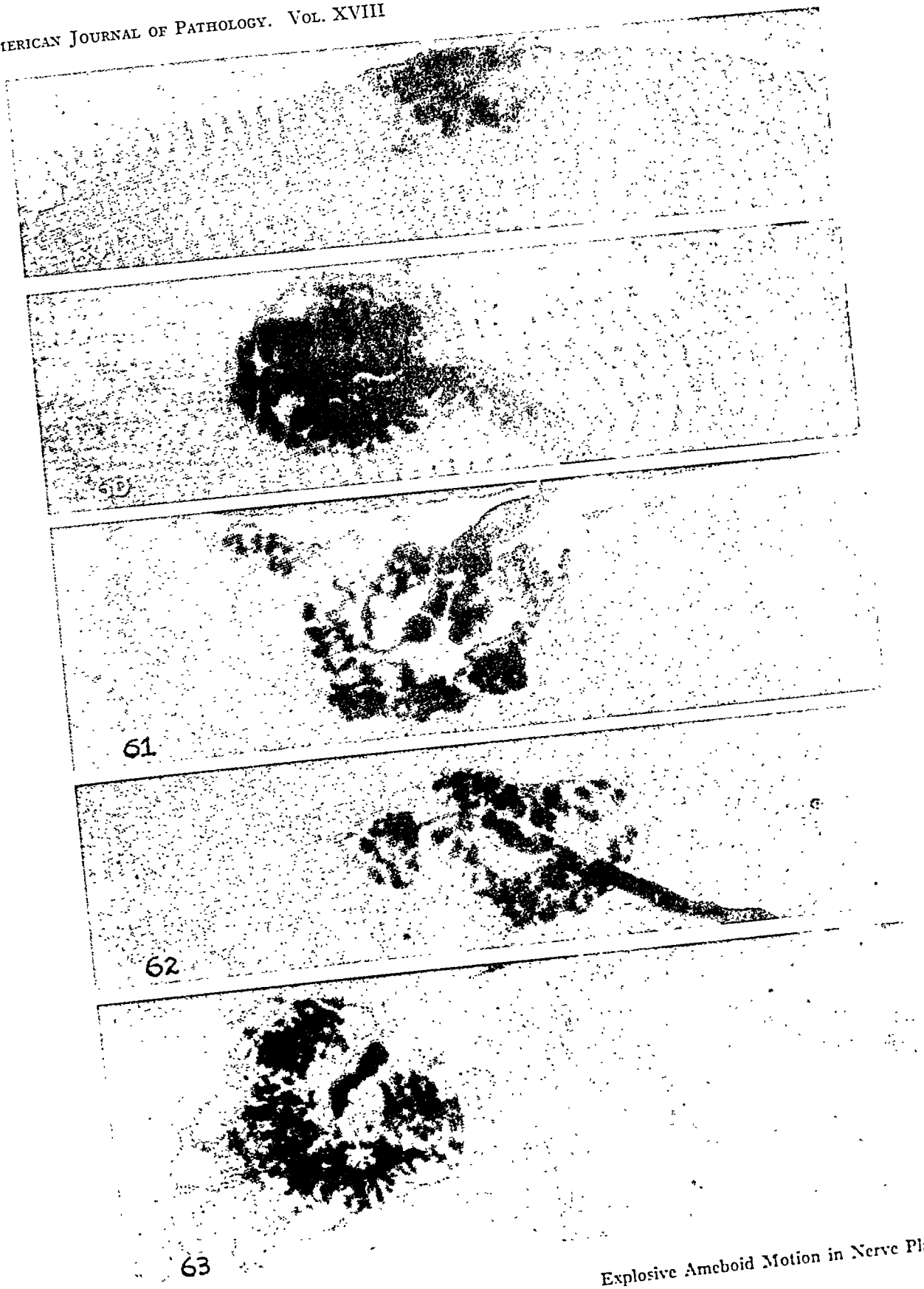


Explosive Ameboid Motion in Nerve Plates

PLATE 50

FIGS. 59 to 63. Pleomorphism of the motor nerve plates produced by superfunctional ameboidism in response to a lethal amount of electrical stimulation in the right intercostal muscle fibers. The retracted motor nerve plate with coarse fronds (Fig. 59) has 14 broad, dark cross striations in close relation to it, whereas the expanded nerve plate (Fig. 63) has 31 fine, dark cross striations in close relation. There is a clear halo-like zone between the coarse fronds of the retracted axonic bulb (Fig. 59) and the dark, granular sole plate of Kühne. The ultra-terminal collateral is observed (Fig. 62) as a fine, non-medullated branch of the axonal ramification in the typical expanded somatic motor nerve plate and ends in the same muscle fiber a short distance to the left from the plate of origin. There is a terminal collateral which arises from the axon a short distance before it penetrates the sarcolemma and ends as an independent small plate above the main nerve plate (Fig. 61). There is a replacement of the regular striations by an irregular granulation. Nucleated ovoid clear spaces, like vacuoles, occur around the periphery of the motor nerve plate and are projected along a granular stream-line in the muscle substance to the right of the nerve plate (Fig. 63). The unipolar expansion of the motor nerve plate toward the left (Fig. 60) results in fine striae and irregular granulation of muscle substance toward the left in the direction of nerve plate expansion. Toward the right the striae are coarse and irregularly placed. The retraction of the pseudopodia of axonic divisions toward the base of the stem forms a wide terminal bulb like a growth cone. The lobes of the retracted bulb are short and wide radicals. There is a massive transmission of nerve substance into the axonal ramifications in the expanded motor nerve plates, stimulated by electricity.

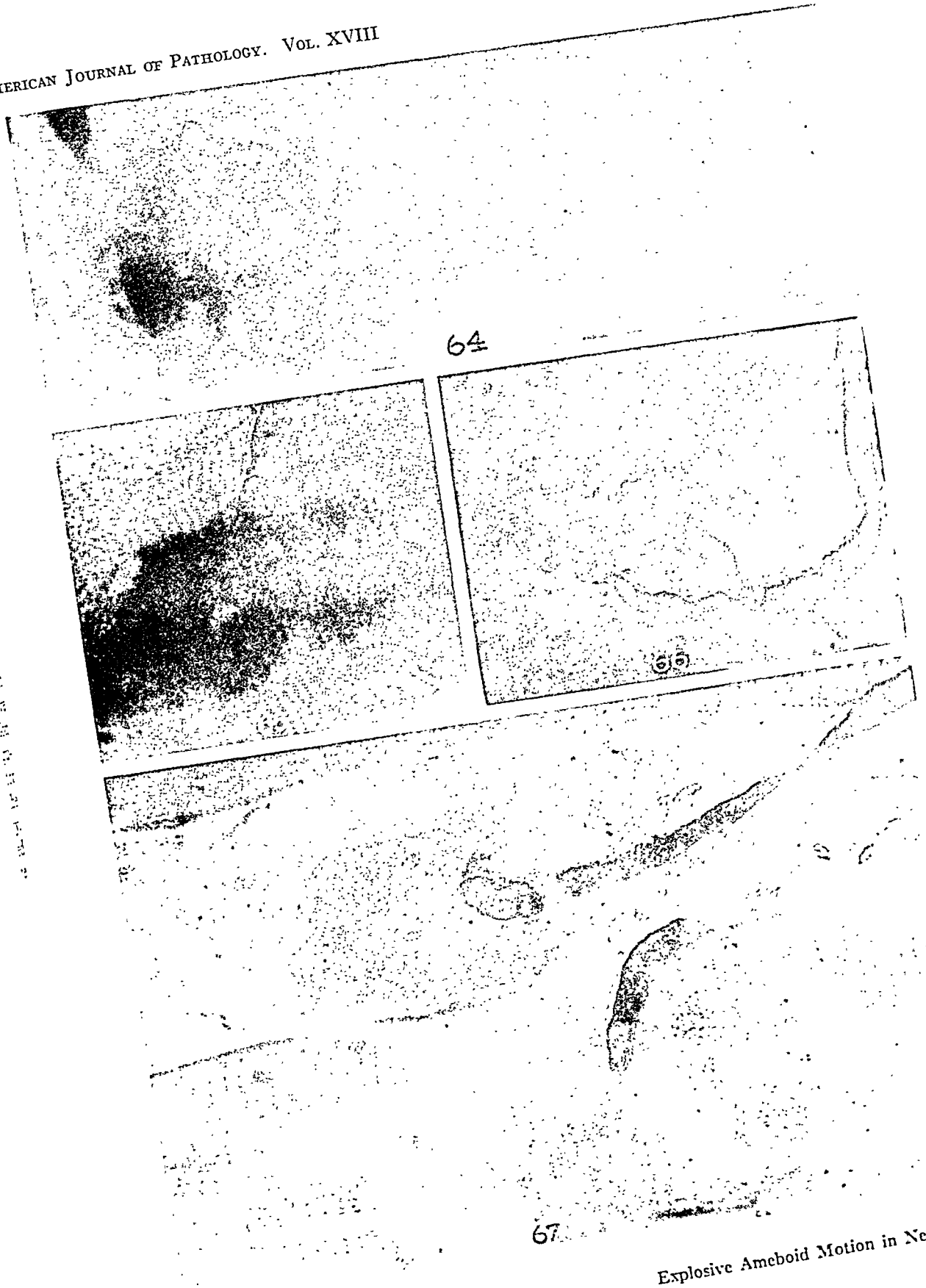
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Explosive Ameboid Motion in Nerve Plates

PLATE 51

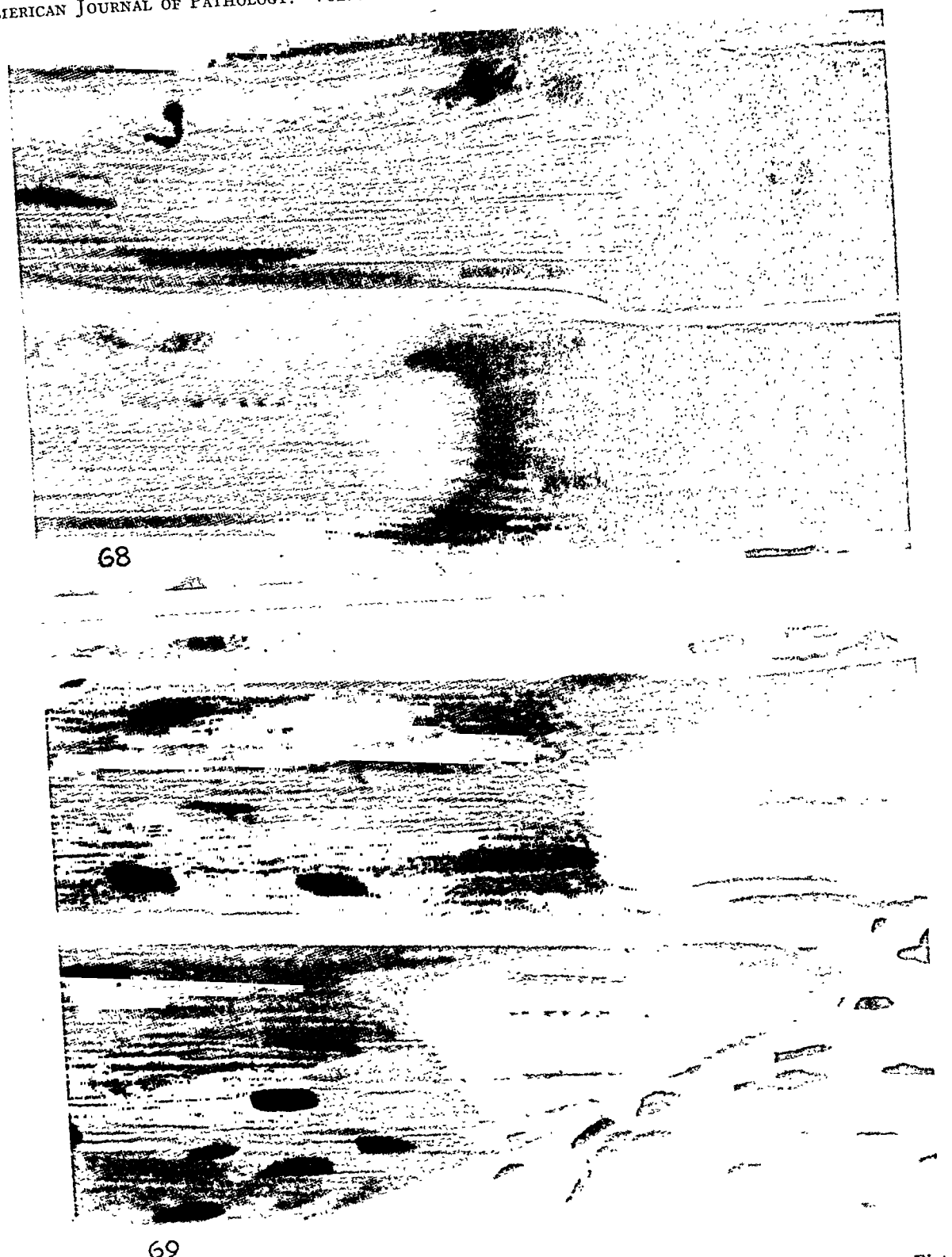
FIGS. 64 to 67. Pleomorphism by superfunctional and explosive ameboidism; teased right intercostal muscle, stimulated by a lethal alternating current, 120 volts, 1 ampere, for 1 minute. The expanded motor nerve plates have variable relationships to the muscle structure. There is a partial obliteration by a granular streamlining of the cross striations radiating with their convexities to the right of the muscle fiber (Fig. 64). There are fine closely spaced striae related to the kidney-shaped nerve plate (Fig. 65), and an obliteration of the striae related to the nerve plate (Fig. 66) which is supplied by two branches after the single axon undergoes dichotomous division. There is a complete explosive disruption, dissolution, and granular replacement of the arborizations of the axons in the two neighboring motor nerve plates (Fig. 67). This condition is found in 0.5 per cent of the motor nerve ends. There is likewise a replacement of the periodic pattern of muscle striae in this area of sudden and explosive disappearance of the two motor nerve plates by granular disorganization through strong electric stimulation. The nucleated ovoid clear areas are irregularly scattered in the myoplasm surrounding the ghostlike outline of the motor nerve plates. There is a massive transportation of nerve substance into the axonal branches in the expanded motor nerve plates, stimulated by electricity. There is an accompanying perturbation of the related protoplasm of the striped muscle fiber. The surface area of the strongly stimulated motor nerve plate is greatly increased over that of the resting nerve plate.



Explosive Ameboid Motion in Nerve Plates

PLATE 52

FIGS. 68 and 69. Photographs of longitudinal sections of rectus abdominis muscle of winter frog, after electrocution by alternating electric current, 120 volts, 1 ampere, for 1 minute, with one electrode in the cervical, the other in the lumbar region. The muscle fibers of the winter frog have central nuclei. The deformations of the relatively passive nuclei, therefore, in the myoplasmic nodes and internodes of electric contracture are definitely portrayed. The nodes are condensed with fine, closely spaced cross striations. The internodes are rarefied with coarse, widely spaced striae or in some places with complete absence of striae. The correlative changes of the nuclei are clearly evident. They are rounded ovoid and compressed in the nodes of condensation; and are elongated or stretched in the internodes of rarefaction. The nodes and internodes of strong contracture are alternately spaced within the single muscle fiber. The myoplasm is more definitely fibrillated in the internodes. These zones of condensation and rarefaction of contracture of the muscle fiber are assumed to be the two components of the longitudinal compression wave which accompanies both the normal and abnormal changes in capillary chemistry in the microcapillary muscle fiber. Hematoxylin and erythrosin stain. × 750.



Explosive Ameboid Motion in Nerve Plates

Carey

INFLUENCE OF COLCHICINE DURING METHYLCHOLANTHRENE EPIDERMAL CARCINOGENESIS IN MICE*

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A marked reduction in intranuclear viscosity during experimental epidermal carcinogenesis in mice was reported in an earlier paper (Cowdry and Paletta,[1] 1941). It was assumed that when two tissues are subjected to equal ultracentrifugal force, the intranuclear viscosity is less in the one in which the displacement of nucleoli and basophilic chromatin is the greater. Using this material for untreated controls, it seemed desirable to ascertain the influence of colchicine on displaceability in similar tissues, paying particular attention to nuclei in mitosis.

The available evidence that colchicine may have some influence on the viscosity of dividing cells is difficult to evaluate. That alterations in viscosity do occur during mitosis in cells not under the influence of colchicine has been known for a long time. The early literature on the subject has been well summarized by Wilson[2] (1925). It deals, however, chiefly with the eggs of invertebrates, mostly sea urchins, and it is not safe to conclude that similar changes take place in the mammalian cells with which we are concerned, for in their case the stratification of contents requires much greater centrifugal force. But it may be significant for us that Heilbrunn[3] (1920) has stated that in sea urchin eggs (*Arbacia*) the appearance of the spindle depends absolutely on a preliminary cytoplasmic gelation and that suppression of gelation results in every case in suppression of the spindle. The point is that Ludford[4] (1936), on the basis of extensive experience with colchicine, has expressed the opinion that this alkaloid brings about some alteration in the physical state of the mammalian cells which he studied and further that it may prevent spindle formation. If Heilbrunn's conclusion can be extended to include mammalian cells, it would be logical to expect suppression of spindle formation by colchicine in them to be occasioned by failure in gelation, or, expressed differently, by an inhibition of expected increase in viscosity, because Wilbur[5] (1940) has found in *Arbacia* eggs that the increase in viscosity which normally follows fertilization is inhibited by colchicine in a concentration of 1 part to 2000.

* Aided by grants from the National Cancer Institute, and from an anonymous donor.
Received for publication, July 23, 1941.

Experiments

The mice used in these experiments each received a single subcutaneous injection of 0.01 per cent colchicine, the amount being 0.25 cc. per 20 gm. of body weight. The animals included late embryos and adults, treated with methylcholanthrene as previously described (Cowdry and Paletta,[1] 1941). Care was taken to subject all of the tissues to the same ultracentrifugal force and to employ the same routine histological technic.

The material is summarized in Table I. In the first column are given the nature and the source of the material. The periods of treatment with methylcholanthrene are given in the second column and the

Table I
Experiments

Tissue	Period of methylcholanthrene treatment in days	Hours after colchicine injection	Strains of mice
Embryonic epidermis			
Back			
9 mm. embryo	0	5	Swiss
11 mm. embryo	0	5	Swiss
18 mm. embryo	0	4	New Buffalo
20 mm. embryo	0	5	Swiss
24 mm. embryo	0	5	Swiss
Hyperplastic epidermis			
Back	22	4	Strain A
Back	22	4	Strain A
Ear	55	4	Strain A
Back	75	5	Swiss
Back	75	5	Swiss
Squamous-celled carcinoma			
Back	71	1, 2, 3, 4, 5, 6, 7	Strain A
Back	71	4, 70	Strain A
Back	82	3	New Buffalo
Back	84	0, 5, 26	New Buffalo
Back	98	0, 5, 48	New Buffalo
Back	99	0, 5, 43	New Buffalo
Back	99	0, 5, 43	New Buffalo
Back	110	1	New Buffalo
Back	110	3	New Buffalo
Back	110	6	New Buffalo
Back	110	8	New Buffalo
Back	110	0, 5, 48	New Buffalo
Back	130	1	New Buffalo
Back	130	3	New Buffalo
Back	130	7	New Buffalo
Back	130	4, 24	New Buffalo
Back	130	4, 24	Swiss
Back	140	1	New Buffalo
Back	140	5	New Buffalo
Back	147	1, 24	Swiss
Back	147	2, 24	Swiss
Back	147	6, 24	Swiss
Back	150	2	Swiss
Back	150	10	Swiss
Back	161	4	New Buffalo

hours after the injection of colchicine in the third. From a squamous-celled carcinoma of 71 days, seven biopsy specimens were taken at hourly intervals. From some, specimens were excised before the injection of colchicine, at 0 hours, but this interfered with the blood supply and the subsequent distribution of colchicine to the tissue so that other specimens collected later on from the same tumor did not show uniformity in colchicine effect.

In addition to the routine technic of Bouin fixation and hematoxylin and eosin staining, other methods were employed in special cases: (1) the Feulgen reaction for thymonucleic acid (Cowdry,[6] 1928); (2) fixation in Regaud's fluid, mordanting in potassium bichromate solution and staining with aniline fuchsin and methyl green for mitochondria; (3) micro-incineration for mineral constituents (Scott,[7] 1937).

Observations

Embryonic Hair Follicles

The change produced by colchicine is more conveniently studied in the hair follicles than in the epidermis itself because of the larger number of mitoses in the former.

Figure 1 shows the epidermis in the upper portion of the field and below a portion of a hair follicle from an embryo, of which the mother received a subcutaneous injection of colchicine 5 hours before its removal and preservation. Five arrested mitoses are included. They are all prophases or early metaphases but in other parts of the specimen a few anaphases and telophases were found. The uniformity of the clumping of the chromosomes is remarkable. The density of the clumps is less than in squamous-celled carcinoma. Comparison of Figure 1 with Figures 7, 8, 11 and 12 illustrating carcinoma cells 5 hours after colchicine injection shows that in the latter the chromosome clumps are without any visible internal organization, whereas in Figure 1 they are less black, and paler areas can be seen within them, indicative of the inclusion of nonchromosomal material (ground substance) and therefore of less dense packing of chromosomes. Moreover, the outlines of the chromosome clumps are definitely smoother in the case of the carcinomatous mitoses, likewise suggesting a closer packing or agglutination. The slightly granular cytoplasm of the embryonic mitoses stains faintly and is small in amount owing to the high nucleo-cytoplasmic ratio.

A portion of epidermis, adjacent to that represented in Figure 1, is illustrated in Figure 2 as it appeared after centrifugation. In the hair follicle in the lower part of the figure, two chromosome clumps have been displaced centrifugally and the areas of cytoplasm, which they originally occupied, have been left behind and appear clear. Small

amounts of stainable cytoplasm can be distinguished. This shifting of chromosome clumps is quite uniform. Their displacement is slightly greater than in control, uncolchicinized tissues but the difference is not easily measured quantitatively.

Hyperplastic Hair Follicles

Figure 3 shows many mitoses arrested by colchicine. The degree of clumping of chromosomes into masses is fairly uniform. The masses are denser than in colchicinized embryonic hair follicles. They appear solid black in the photomicrograph and are therefore more compact than those in Figure 1. Moreover, their outlines are smoother and the ratio of the volume of clump to volume of cytoplasm is less than in Figure 1. This is interesting because, judging from the figures, the nucleocytoplasmic ratio in nondividing cells is not noticeably greater than in the embryonic follicle. Either the cytoplasm of each of the cells in arrested mitosis in Figure 3 took up water or its apparent area is increased by a closer packing of the chromosomes into smaller masses. The fact that the cytoplasm seems clearer in Figure 3 may indicate a difference from Figure 1 or it may be a photographic artefact.

On centrifugation the chromosome masses of colchicinized hyperplastic hair follicles are slightly displaced (Fig. 4); but the displacement is neither so great nor so uniform as in the colchicinized embryonic follicles (Fig. 2). The clumps are larger and less dense than those in colchicinized hyperplastic follicles (Fig. 3). In control, hyperplastic follicles not subjected to colchicine, the displaceability of the chromosome clumps was about the same as in the colchicinized ones. The stainable cytoplasm is slightly displaced (Fig. 4). The chromosome clumps in the few arrested mitoses encountered in the hyperplastic epidermis itself showed much less displacement than those in the hyperplastic hair follicles.

Squamous Cell Carcinomata

The first evidence of colchicine influence was observed 1 hour after injection of the alkaloid, though it may have occurred earlier. It reached a maximum between 4 and 5 hours, was maintained for about 12 hours and subsided in the interval between 12 and 24 hours. The number of arrested mitoses depends upon the rapidity of growth of the tumor and the adequacy of the blood supply bringing in the colchicine. The time from beginning to end of a normal (untreated) epidermal mitosis is not known.

Figures 5 and 6 illustrate early (3 hours) and late (12 hours) stages in the response. In both of the specimens, however, there was

variability and it is not to be supposed that all of the arrested mitoses are like those included in the photomicrographs. These two figures are presented to illustrate some features in the structure of the chromosome clumps and of the clear areas about them in colchicinized carcinomata which have not been centrifuged.

Two mitoses are shown in Figure 5. In the upper one, not fully contained in the section, individual chromosomes can be distinguished but they look slightly swollen and appear to stick together. They are surrounded by thin halos of lightly staining cytoplasm. In the lower, the chromosomes are so closely agglutinated that in the center of the mass no structural details can be made out. The periphery of the mass appears crenated and it is possible that some of the bulgings may represent the surfaces of chromosomes. About the mass there is a more extensive clear area.

Figure 6 shows an arrested mitosis of a large edematous cell. The outlines of the chromosome clump are almost spherical and its surface smooth. The agglutination of chromosomes has progressed farther than in the lower mitosis of Figure 5. The clear area is very much larger and more sharply separated from its environment of rarefied cytoplasm. We shall now describe in a more detailed way the chromosome clumps, clear areas and surrounding cytoplasm.

CHROMOSOME CLUMPS

That the majority of these large, single chromosome clumps are contained in mitoses which have not progressed beyond the metaphase is clear. It is, however, more difficult in colchicinized methylcholanthrene carcinomata to state whether the clumps represent arrests in the prophase or in the metaphase than in hyperplastic or embryonic hair follicles because of the greater density of the chromosome clumps. Often the chromosomes are so close together that longitudinal splitting, if it occurred, would be obscured and equatorial plate formation, if it took place, would be lost by the clumps taking the shape of rough spheres.

A conspicuous feature of the chromosome clumps of late prophases or metaphases is that some of them can be dislodged by the sweep of the knife in the cutting of sections. Figure 7 shows part of a section of a centrifuged squamous-celled carcinoma 140 days after beginning treatment of the epidermis with methylcholanthrene and 5 hours after the injection of colchicine. Two mitoses are included. In the one situated on the right, about halfway between the upper and lower margins of the figure, the chromosome mass appears black, is circular in cross section and is somewhat smaller than the neighboring nuclei. The mass is displaced centrifugally (downward in the figure) so that it

comes in contact with the cell membrane. The basophilic chromatin in the non-dividing nuclei is displaced in the same direction. The one on the left is at about the junction of the upper and middle thirds of the figure. As in the first, the chromosome mass appears solid black; but it has been forced part or all of the way out of the cell in which the space which it formerly occupied can be seen. A wavy line in the cytoplasm may represent part of the nuclear membrane left behind. Such differences in degree of displacement are not unusual.

Figure 8 presents a section from the same block of tissue as Figure 7, but cut in the reverse direction. The sweep of the knife was upward (in the figure) against the direction of centrifugal force. In the upper right a chromosome mass is recognizable by its solid black appearance and slightly crenated margin. It has been carried by the knife out of the position it formerly occupied and which now appears empty. The mass has been pressed against the cell membrane but has not lost its shape. In the three nuclei above it the basophilic chromatin has been shifted in the opposite direction (centrifugally).

In rare cases chromosome masses have been carried completely out of their cells of origin by the knife; but the great majority of them are not displaced by sectioning. Why some are taken while others are left, we are unable to explain. That both look alike can be seen by comparing a displaced one (right in Fig. 7) with an undisplaced one (lower left part of Fig. 8). Examination of sections of 25 uncolchicinized squamous-celled carcinomata did not bring to light a single instance of dislocation of chromosome masses by the microtome knife. The tightening up of chromosomes into such masses, under the influence of colchicine, is more marked in malignant cells than in embryonic or hyperplastic ones.

Figures 9 and 10 supply a very limited comparison of the effect of centrifugation on untreated and colchicinized carcinomata respectively. In the first the displacement of basophilic chromatin and nucleoli in nondividing cells is much greater than in the second. Two chromosome masses are included in the first, one of them partly hidden by the number 9. The other is above it and is somewhat flattened against the cell membrane. Both are much displaced. A single and larger chromosome mass is shown in Figure 10. This is also displaced and is perhaps a little flattened. But other pairs of comparable cells in carcinomata, chosen because the members of each pair appeared to be similar and had been subjected to as nearly as possible the same centrifugal force, provided apparent examples of the reverse result; that is, of an increase in displaceability under colchicine. Still other pairs seemed to indicate that the colchicine was without influence on displaceability.

Attempts were made to make comparisons with tissue from the same tumor. First, a piece was removed and centrifuged. Then, colchicine was injected into the animal and finally the rest of the tumor was excised and centrifuged in the same way. The results were disappointing. The first excision apparently interfered with the blood supply and prevented the colchicine from adequately affecting the remainder of the tumor. That the comparison of treated and untreated neoplasms must be between areas having about the same blood supply is clear from this experience as well as from the observation of Brues and Jackson [8] (1937) that, 10 hours after injection, colchicine does not act in the deeper part of mouse sarcoma 180.

Comparison of tumors removed at various times after injection of colchicine and immediately centrifuged with similar tumors not colchicinized did not settle the question. Each tumor has a more or less pronounced individuality so that the degree of similarity in any comparison was seldom sufficient. And within each there is confusing regional diversity in mitotic frequency, cellular differentiation and degeneration. At present, therefore, we are unable to state whether the displaceability of chromosome masses in squamous-celled carcinomata by centrifugal force is influenced by colchicinization. We think, however, that if there is any influence on displaceability it is not very definite or uniform.

One would look for an increase in the displaceability of chromosome masses by colchicine because, in squamous-celled carcinomata as well as in embryonic and hyperplastic hair follicles, colchicine increases the density of the clumps of chromosomes. But, by the phrase "increase in density," only a reduction in space between the chromosomes is meant, in consequence of which they become more closely or densely crowded together so that in many cases their individual outlines cannot be resolved.

That the clump stains more homogeneously is not sufficient evidence of greater specific gravity. As already stated, some chromosomes in colchicinized tissues are noticeably swollen. If many of them swell, their total volume may be greater even though the area occupied may be less by reason of the smaller amount of ground substance included in the mass.

If the swelling is due to an increase in substance of the same kind, the specific gravity would remain the same. In an earlier paper we found a progressive increase in nuclear and cytoplasmic volumes during carcinogenesis (Cowdry and Paletta,[9] 1941). There is more chromatin to clump. Whether the number of chromosomes in each mitosis has been increased or not will have little effect on the density unless

they undergo some chemical change. If, on the other hand, the swelling is occasioned by intake of water—a possibility indicated by the fact that the centers of the swollen chromosomes sometimes stain less intensely than their peripheral parts—a decrease in specific gravity might be brought about.

The fact that some chromosome masses in colchicinized tissues offer more resistance to the edge of the microtome knife than the aggregates do in uncolchicinized tissues suggests greater density, in the sense explained above, in their fixed, dehydrated, cleared and paraffin-embedded state but not necessarily greater specific gravity *in vivo*. The Feulgen reaction for thymonucleic acid is often slightly different after colchicinization. The chromosome clumps assume a bright cherry red color in contrast with the usual purple color.

After micro-incineration the mineral residues of chromosome clumps in mitoses arrested by colchicine can be identified and in many cases can be distinguished from those of the less dense clumps in uncolchicinized carcinomata. Viewed in the dark field, there is a greater range in size in the light-reflecting granules of the former. The proportion of bright white granules of Ca and/or Mg seems to be increased and that of fine bluish white granules of Na and/or K decreased. Whether there is a change in the red granules of Fe could not be ascertained. Neither could it be discovered whether the total amount of ash of the clumps revealed by this method is modified by the colchicine. But, after colchicine, the ash appeared to be somewhat less well organized in space; in other words, it was not so regular. However, the comparison was only visual and it is particularly difficult in micro-incinerated specimens to so arrange the experiment that the areas of tissue compared are similar. If the comparisons had embraced a large number of better selected tissues, cases might have been found in which the differences referred to were absent.

CLEAR AREAS

Since no adequate search was made for clear areas about the chromosome clumps in living epidermal cells, conclusions as to their nature must be very tentative.

The small halos about individual chromosomes (Fig. 5) may be optical artefacts caused by the refraction of light from surfaces, but the clear area about the upper chromosome mass in Figure 12 is certainly too large to be an optical artefact.

The possibility that the halos are in part artefacts of fixation cannot be altogether excluded because, though the chromosomes are swollen, their size may have been still larger before fixation. In the coagulation caused by the fixative they may have lost fluid, which may have con-

tributed to the formation of the halos. The halos are seen after fixation in the fluids of Bouin, Helly and Regaud; but, of these three, they were most conspicuous after Bouin's fluid.

In centrifuged tissues the clear area is ordinarily of about the same width on all sides of the chromosome clump. Its size seems, in some cases, to bear a relation to the size of the chromosome clump. Thus it is large in Figure 6 and small in Figure 13. But it is not unusual to find clear areas of different sizes about chromosome clumps of approximately the same size (Figs. 12 and 13).

The line of demarcation between the clear area and the surrounding stainable cytoplasm is often distinct (Fig. 6). It may even be membrane-like and stain faintly with eosin or hematoxylin. Thus, the clear area about the lower chromosome clump in Figure 12 appears as if surrounded by a slightly crumpled, nuclear membrane. When specimens like that illustrated in Figure 6 are incinerated, the surface of separation is studded by a mixture of small bluish white and flat white mineral residues suggestive of a nuclear membrane, but the clear area itself is devoid of demonstrable minerals. Another point suggesting, but not proving, that in some instances the line of separation is a nuclear membrane, is the absence of mitochondria within the clear area limited by it, for mitochondria are never within nuclei. When, however, the clear area is small and indistinct, it grades imperceptibly into the cytoplasmic environment and is obviously not limited by a nuclear membrane.

In centrifuged specimens, in which the chromosome clumps have been displaced, the clear areas may wholly encircle them (Fig. 12), partly surround them (Fig. 14) or they may be limited chiefly to their centripetal sides (Figs. 7 and 10), which is inconsistent with their interpretation as artefacts of fixation. Centrifugation does not lead to a suppression of the clear areas by a shifting of stainable cytoplasm into them. The often very sharp margins between clear areas and stainable cytoplasm remain (Fig. 10).

The evidence at hand seems to indicate, therefore, that, when influenced by colchicine, the chromosomes clump together in a dense mass. They withdraw themselves to some extent from the surrounding substance and leave a clear area which is intranuclear and more conspicuous in those cases in which a nuclear membrane persists even as a vestige.

SURROUNDING CYTOPLASM

The stainable cytoplasm of colchicinized cancer cells is not always of the same density. Its appearance depends somewhat upon the fixation but there may be a definite hydropic change. The displaceability

of the stainable cytoplasm frequently is less when it is more hydropic, or rarefied, as may be seen by comparing Figure 12 with Figure 11. In some cells even the chromosome clumps are not displaced (Fig. 13). Particularly is this the case in those whose cytoplasm is strongly acidophilic when stained by hematoxylin and eosin. The affinity for eosin of cytoplasm in intestinal cells of mice 8 hours after the injection of colchicine was noted by Clearkin [10] (1937). Centrosomes and spindle fibers were not observed by us, but a special search was not made for them in iron hematoxylin preparations appropriately counterstained.

In preparations for the demonstration of mitochondria it is a simple matter to select a number of mitoses which, from the appearance of their chromatin masses, seem to have been equally arrested by the colchicine. But in them the properties of the mitochondria are not uniform. In some the mitochondria have the usual rodlike and filamentous shape while in others they have been changed into deeply staining spherules which may be of variable size and of uneven distribution in the cytoplasm. In still others the mitochondria are represented only by fairly large vesicles with more or less clear centers. There is, in other words, a gradation from mitochondrial contents that appear to be normal, to others indicative of injury and of death, or of approaching death. We are unwilling to commit ourselves as to how far spherule and vesicle formation can proceed while the possibility of reversion to normality remains.

That these differences between the mitochondrial contents of arrested mitoses are not due to variations in technic is evident from the fact that they occur in the same preparation in cells equidistant from the surface of the tissue. There are two other possibilities. The mitochondrial changes are either a primary result of colchicine action or a secondary result in consequence of the mitotic arrest. It is conceivable that the mitochondria are most altered in the mitoses held longest in the metaphase. Perhaps some of the arrested mitoses do not survive and the mitochondria are cytoplasmic indications of serious injury which is not marked by further detectable alterations in the chromosome masses.

Discussion

In the tissues examined and under the conditions specified, colchicinization renders the chromosome masses slightly more displaceable by centrifugal force in embryonic hair follicles but no consistent difference was noted in their displaceability under colchicine in hyperplastic hair follicles and in squamous-celled carcinomata in the methylcholanthrene series.

It is possible that, in the embryonic hair follicles which were not

subjected to methylcholanthrene, the increase in displaceability on colchicinization was caused by a decrease in viscosity of the ground substance. But the chromosomes in the mitoses arrested by colchicine differed from those not under colchicine. They were more closely packed together. Whether this difference is accompanied by an alteration in their specific gravity we do not know; but if their specific gravity is increased relative to that of the ground substance, their tendency to displacement would also be increased because the push, or the pull, exercised by the centrifugal force would be greater.

Conditions in the tissues previously treated with methylcholanthrene are more complicated. In them the density of the chromosome clumps is greater than in the embryonic follicles to which the carcinogen was not applied, especially in the carcinomata which were subjected to it over a longer period. Some of the clumps in the carcinomata offered more resistance to the sweep of the microtome knife, were colored a brighter cherry red by the Feulgen reaction and exhibited a different mineral residue on micro-incineration than those in either the hyperplastic or embryonic follicles. In addition the ground substance in the arrested mitoses differed from that in mitoses not under the influence of colchicine. It was divisible into characteristic clear areas about the chromosome clumps and into investing cytoplasm, the mitochondrial content of which was modified. Consequently, the possibility of changes in specific gravities under colchicine, which might partly condition displaceability by centrifugal force, is considerably enhanced; but there is no evidence that the differences mentioned are actually correlated with alterations in specific gravity.

If, however, changes in the specific gravities of chromosomes, clear areas and cytoplasm are produced by colchicine, the fact that displaceability is not significantly altered does not necessarily mean that the colchicine is without influence on viscosity. Obviously, an increase in viscosity of the ground substance of the mitoses might not result in lesser displaceability of the components if their specific gravities were so increased that they became more susceptible to displacement by the centrifugal force. It is equally evident that a decrease in viscosity of the ground substance might not result in greater displaceability if their specific gravities were so decreased that they became less susceptible to displacement. Our experiments with the ultracentrifuge therefore do not indicate either a change or an absence of change in viscosity of the ground substance of mitoses arrested by colchicine in hyperplastic hair follicles and carcinomata.

It is doubtful whether the larger of the clear areas which we have described about the chromosome clumps are similar to those reported

by Ludford [4] as occurring in Flemming preparations stained with iron hematoxylin and which he thought might represent spindle substance. Evidence is altogether lacking that the clear areas in our specimens are composed of spindle substance. Their chief characteristics are lack of stainability and absence of detectable mineral constituents. Their shape is in no way suggestive of spindles, but they occur near the chromosome masses. This is the normal site of spindle development. A feature of the clear areas is that they tend to hold their shape and do not merge with the stainable cytoplasm on ultracentrifugation. They may be areas of gelation or of increased viscosity; but, if this is the case, the absence or rarity of spindle formation reported by Ludford and others would not be correlated with a failure of gelation—a possibility mentioned in the introduction to this paper.

Heilbrunn [3] has mentioned abstraction of water as one cause of gelation in *Arbacia* eggs. Other things being equal, loss of water would bring about a decrease in volume. Apparently no one has made detailed measurements of normal and colchicinized mitoses to ascertain whether a change in volume actually does take place, but Allen, Smith and Gardner [11] (1937) noted that mitoses under colchicine are of large size and round or oval shape. In our preparations the cells in mitosis are rounded and some (Fig. 6) appear to be swollen. If the arrested cells actually become more watery, it would not be surprising to discover a decrease in viscosity; but a change in viscosity can occur without change in volume by redistribution of water, as when a colloidal solution gels. The possibility that colchicine in some cases facilitates the entry of water into mitoses is interesting from another angle since it may explain why tumors under the influence of colchicine are more susceptible to the destructive action of distilled water, as has been reported by Guyer and Claus [12] (1940).

According to Ludford,[4] the prophase is the phase of mitosis least affected by colchicine because the nuclear membrane is impermeable to it. Our observations of clumped chromosomes in mitoses provided with what looked like nuclear membranes, may not be of significance in this connection for such membranes were seen only in the carcinomata in which the range of variation of the cells was much wider than in the embryonic or hyperplastic hair follicles. Indeed, the nuclear membranes in question may have become so altered that colchicine could get in. Since Scott [13] (1936) has been successful in the analysis for minerals of nuclei separated from cytoplasm and collected in fair quantity, it may be feasible, in a later study, to determine whether colchicine can enter nuclei. Another test would be to ascertain whether colchicine arrests mitoses in those forms, mentioned by Wilson,[2] in

which the nuclear membrane persists throughout the whole process of mitosis.

Like others, we have found some variability in the appearance of arrested mitoses. This effect was most marked in the methylcholanthrene carcinomata, less in the methylcholanthrene hyperplasia and least in the embryonic hair follicles. Despite a certain range of variation in almost any specimen, it is appropriate to recognize two types of arrest. The first is the "condensed arrest," described in this paper and in many others, in which the chromosomes are drawn together or "contracted" into a clump. The second may be called "dispersed arrest." The complete scattering of chromosomes described by Brues and Jackson [8] as a first stage in the reaction of regenerative (compensatory) mitoses in the livers of rats to colchicine comes under this heading. Not only the first stage but, as far as the information goes, the whole process resembles the "forme chromatorhexique" of arrest reported by Dustin and Zylberszac [14] (1939) in compensatory mitoses in the kidney. A similar dispersal of chromosomes and of small chromosome clumps was described by Dustin and Chodkowski [15] (1938) in endothelial cells in colchicinized cicatrizations, but, judging from the figures of some of the arrests, the chromosomes were condensed, not dispersed. Perhaps the two types may exist side by side in the same tissue. Dustin and Zylberszac suggest that the "forme chromatorhexique" is occasioned by strong concentration of the poison in organs like the liver and kidney which eliminate it. But it is unsafe to assume that mitoses in different tissues in normal growth, as well as in compensatory hypertrophy, respond equally to equal concentrations.

Quite apart from the relationship between the two types of arrest, it is evident that colchicine can bring about either a condensation or a dispersal of chromosomes. The physical forces operating would appear to be different in the two cases. Ultracentrifugation and micro-incineration of the dispersed arrests might yield entirely different results from those obtained by us with contracted arrests.

To predict the action of colchicine on any particular tissue from descriptions, however accurate, of its influence on other tissues is to court mistakes. We have found slight differences in its action on epidermis and hair follicles of mice, and Carleton [16] (1939) noted a difference in the response of the skin to colchicine in rats of the same strain and even of the same age, but she did not refer to any difference between sexes. The claim of Havas and Gal [17] (1938) that methylcholanthrene sensitizes a great number of cells to colchicine is neither supported nor contradicted by our experiments, since we made no counts, but the chromosome clumps in the contracted arrests in our

methylcholanthrene-treated tissues were denser than in untreated tissues. Other sensitizers and inhibitors may lurk in the background. The evidence is strong that in some cases colchicine is a mitotic stimulant increasing the number of mitoses (Paff,[18] 1939, and others). It is equally convincing that in other instances colchicine is a mitotic inhibitor decreasing the number of mitoses (Tennant and Liebow,[19] 1940). Failure to find evidence that colchicine acts in either of these two ways is without value except for the particular tissues investigated. For these reasons it seems to us, as to Worthington and Allen [20] (1939), that the validity of using colchicine-arrested mitoses as indices of growth rates depends on proof that the accumulated mitoses are proportionate to those normally occurring in the particular tissue. To prove this point would probably be more complicated and difficult than to estimate growth rates without using colchicine.

Summary

The susceptibility to colchicine of mitoses in embryonic hair follicles, in hair follicles rendered hyperplastic by methylcholanthrene and in squamous-celled carcinomata produced by methylcholanthrene appeared to be slightly different.

In embryonic hair follicles the clumping of chromosomes was less dense than in cancer cells, when both were observed 5 hours after injection of colchicine. The displacement of the clumps by centrifugal force was slightly greater in colchicinized than in uncolchicinized embryonic epidermis.

In hair follicles made hyperplastic by methlycholanthrene, the clumping of chromosomes by colchicine was more marked than in the embryo and less than in cancer cells. The displacement by centrifugal force was less than in embryonic hair follicles and in cancer. No difference was noted between the displacement of the clumps by centrifugal force in colchicinized and uncolchicinized hyperplastic hair follicles.

Squamous-celled carcinomata produced by methylcholanthrene were examined over longer periods of colchicine influence and at more frequent intervals. The chromosomes were more closely packed in clumps than in either the embryonic or the hyperplastic hair follicles. A few of the clumps were dislocated by the sweep of the microtome knife in cutting sections—a phenomenon not observed in 25 uncolchicinized carcinomata in which a search for it was made. Some evidence was found that the thymonucleic acid and mineral contents of the chromosome clumps differ from those of the less compact clumps in uncolchicinized tissues. The chromosome clumps were surrounded by clear areas of variable size. The larger ones were mineral-free in micro-incinerated

sections. The investing cytoplasm varied, as between different mitoses, in its affinity for hematoxylin and eosin and in its mitochondrial content, even when no difference could be distinguished in the chromosome clumps.

The displaceability of the chromosome clumps, clear areas and stainable cytoplasm, under centrifugal force, was less uniform in carcinomata than in the embryonic and hyperplastic hair follicles. No consistent difference in displaceability was observed between colchicinized and uncolchicinized carcinomata. Evidence could not be adduced that colchicine causes a change in the viscosity of mitoses, because alterations in specific gravities may occur, but the possibility that a change in viscosity may take place was not excluded.

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DESCRIPTION OF PLATES

PLATE 53

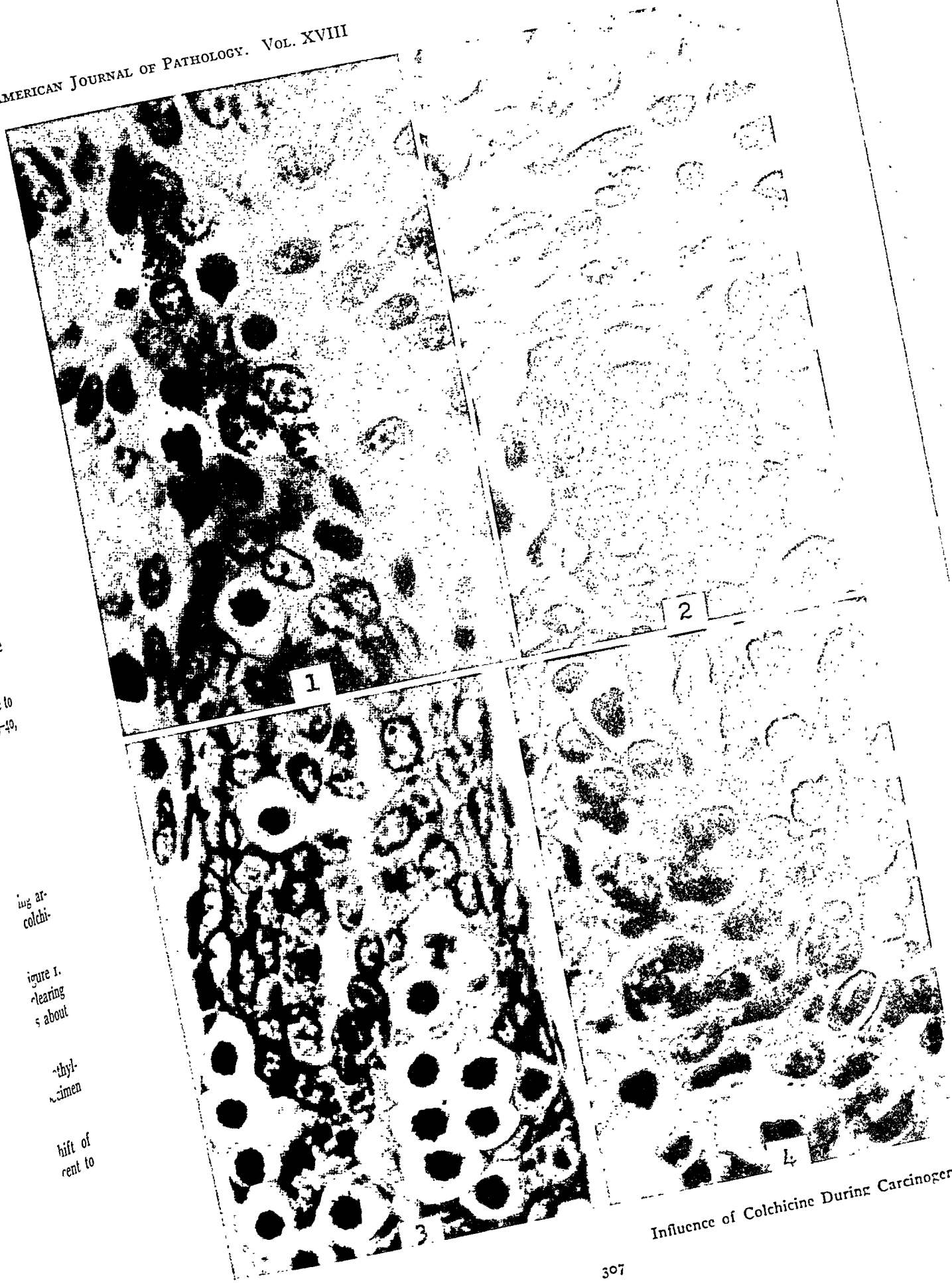
FIG. 1. Epidermis taken from the back of a 20-day mouse embryo, showing arrested mitoses. Specimen obtained 5 hours following the injection of colchicine subcutaneously into the mother's thigh. × 1040.

FIG. 2. Centrifugation of a small piece of skin adjacent to that shown in Figure 1. Marked centrifugal displacement of the chromosomes and centripetal clearing of the cytoplasm are seen. In all centrifuged specimens the force was about 350,000 times gravity for 30 minutes. × 1040.

FIG. 3. Epidermis of a Swiss mouse rendered hyperplastic by painting with methylcholanthrene for 75 days, showing arrested mitoses in hair follicle. Specimen was biopsied 5 hours following the injection of colchicine. × 1040.

FIG. 4. Centrifuged skin of the back of a mouse showing downward shift of chromosomes in arrested mitoses. Specimen obtained from tissue adjacent to that shown in Figure 3. × 1040.

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Influence of Colchicine During Carcinogenesis

PLATE 54

FIG. 5. Cancer cells arrested in division by the action of colchicine. The upper dividing cells show the fragmented appearance of the chromosomes. The lower cell shows a tightly clumped mass of chromosomes surrounded by a dense cytoplasm. Specimen taken from a mouse injected with colchicine 3 hours previously. (Methylcholanthrene, 130 days.) × 1040.

FIG. 6. Large mitosis arrested by the action of colchicine. A clear zone can be seen surrounding the agglutinated chromosomes and the vacuolated cytoplasm. Taken from the same specimen as Figure 5. × 1040.

FIG. 7. Centrifuged tissue from squamous-celled carcinoma 5 hours following the injection of colchicine. The resting cancer cells show displacement of chromatin and nucleoli centrifugally. Chromosomes are seen which were carried out of the cell in the upper left by the cutting knife. (Methylcholanthrene, 140 days.) × 1040.

FIG. 8. Prepared from the same centrifuged specimen as Figure 7, only the tissue was cut in the opposite direction. Here the chromosomes were forced out of the cell in a direction opposite to that shown in Figure 7. × 1040.

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Influence of Colchicine During Carcinogenesis

PLATE 55

FIG. 9. Centrifuged tissue of a squamous-celled carcinoma. This mouse was *not* injected with colchicine. The chromosomes and cytoplasm have shifted to the centrifugal side of the cell. Compare with Figures 10 to 14. (Methylcholanthrene, 166 days.) × 1040.

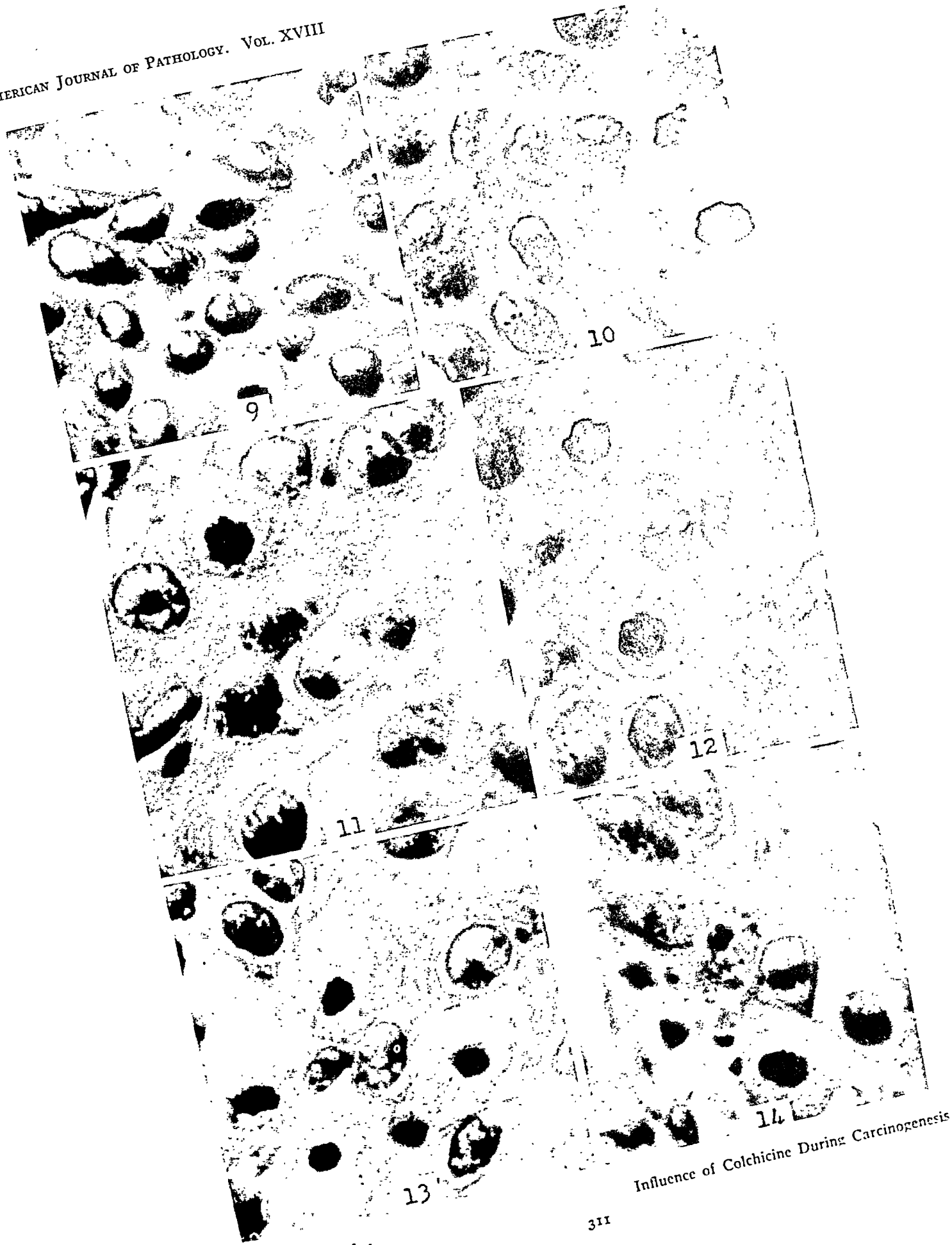
FIG. 10. Centrifuged tissue taken from a squamous-celled carcinoma. This mouse was injected with colchicine 5 hours previously. Although the chromosomes are at the centrifugal side of the cell, the shape of the clear zone and of the displaced cytoplasm behind it has been retained. (Methylcholanthrene, 140 days.) × 1040.

FIGS. 11 and 12. Centrifuged squamous-celled carcinomatous tissue obtained from a mouse which had colchicine injected 5 hours before the biopsy was taken. The shape of the clear area around the chromosome mass has been retained and the shifted cytoplasm does not break through the clear zone. Resting cancer cells show displacement of nucleoli and chromatin centrifugally. (Methylcholanthrene, 140 days.) × 1040.

FIG. 13. Tissue from squamous-celled carcinoma that had been centrifuged after colchicinization for 6 hours. There has been relatively little or no shift of the chromosomes. Compare with Figure 9. (Methylcholanthrene, 147 days.) × 1040.

FIG. 14. Centrifuged squamous-celled carcinoma after colchicinization for 5 hours. (Methylcholanthrene, 140 days.) × 1040.

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SILICOSIS OF SYSTEMIC DISTRIBUTION*

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The present conception of silicosis, in the words of the Committee on Pneumoconiosis and the Committee on Standards of the American Public Health Association, at a joint meeting in 1932, is that it "is a disease due to breathing air containing silica (SiO_2) characterized anatomically by generalized fibrotic changes and the development of miliary nodulation in both lungs."

Except for the associated deposit and similar fibrosis in peribronchial lymph nodes, which is an integral part of the lung disease, there has been no report of free silica deposit and associated anatomical changes in other viscera in naturally occurring silicosis.

Gardner and Cummings [1] have, however, demonstrated that by the injection of particulate free silica directly into the circulation in certain animals a state of experimental silicosis may be produced which is comparable in tissue damage and fibrosis to naturally occurring silicosis in man.

From a postmortem study, herein recorded, it may be concluded that the possible occurrence of silicosis should be widened to encompass the deposit of silica in such locations as the spleen, liver and kidney, with structural changes in at least the first two of these organs. Whether or not this may also entail consequential anatomical damage, especially in the case of the liver, or the possibility of other systemic effects of a toxic nature, can be no more than speculative at the present.

REPORT OF CASE

The following case study is presented as evidence that silica and silicosis are not necessarily confined to the pulmonary area.

The subject was a negro man, 30 years of age, who worked in a sand-drying plant "the majority of his time" for the 6 years prior to his illness. The information came from his physician, who provided the only account of this patient's exposure to dust and of his illness. It was said that in his work he was exposed to very dusty conditions, with no protective measures available, in that he was subjected to the inhalation of fine sand in quantity, blown by heated air.

He was first seen by a physician in March, 1937, and was treated for a fever, judged to be malaria, after which he returned to work for a few days. In April he was seen again, when he complained of fever and general bodily aching. His temperature was then 100° F., his pulse rate was 100 and physical examinati[illegible]d nothing but "occasional inconstant râles" at the bases of both lungs.

* Received for publication, July 3, 1941.

sites were found in the blood, and after antimalarial treatment he returned to work for about 1 week. When seen again in June his condition was apparently the same, save that he stated that he had been coughing considerably and had apparently lost some weight. His temperature was then "slightly elevated" as was his pulse, and râles over both sides of the chest were constant. X-ray examination of the chest at that time was thought to show tuberculosis and he was put on appropriate treatment. The roentgenogram of the chest, seen by me after the autopsy, exhibited rather heavy but irregular shadows in the mid three-fourths or more of the lungs, appearing to follow bronchial markings generally. The apices and bases were comparatively clear. No definite nodules were seen. There was some obscurity laterally near the right apex. The diaphragm appeared high on both sides. The heart borders were obscured by lung densities. When the patient was seen again in November it was said that he gave the impression of an advanced case of tuberculosis, with râles over the entire lung area and friction rubs over both lungs anteriorly and posteriorly. Two months before he died there were signs of complete collapse of the left lung, with friction rubs and râles over the entire right lung area.

Since this man was not under close observation and conditions for thorough clinical study were not available, the clinical record of the case is inadequate. He died in March, 1938, about 1 year after he first became ill, and I made a postmortem examination about 24 hours later under conditions offering minimal facilities. The autopsy protocol (no. 46134–38–56) follows.

Gross Examination

The body was that of a male Negro of rather small stature, quite emaciated, apparently 20 to 30 years of age, in a state of good preservation, having been dead about 24 hours. There were no superficial abnormalities except that the fingernails were bluish, and there was very little fatty tissue. The fingers and toes showed no clubbing.

The right lung was collapsed so far as was possible because of adhesions and solidification. The pleural cavity contained air, the surfaces were shiny and there were a number of heavy pleural adhesions, particularly in the midregion both laterally and posteriorly. The base was free and there was only a small amount of clear brown fluid in the cavity. This lung was comparatively small and in a solidified, contracted state except at the margins and the apex, where, as well as occasionally over the solidified portion, there were air-bearing areas with large emphysematous blebs. The lung sectioned with distinct resistance, being fibrous and apparently gritty. The middle three-fourths, or more, was of a densely solid, dark gray mottled color, the surface being, in general, depressed, with some particularly retracted scars. At the apex the lung tissue was not solid but fibrous strands appeared in it. There were no cavities and no areas of recognizable necrosis.

The peribronchial nodes were enlarged, some to the size of a kidney bean, and had a dark gray marbled appearance.

The left pleural sac had a reddened rough surface, with some deposit

of fibrin upon it, and there were fewer fibrous adhesions than on the right, these being in the midportion and toward the apex but not at it, and laterally and posteriorly. There was about a teacupful of brown flocculent fluid in this cavity. The left lung was very much like the right in the solidified, resistant, gritty, dark gray marbled, densely solid state of the mid three-fourths or more, with depression of the surface and contracted scars in the solid area. There was also air-bearing tissue with large emphysematous bullae in the apex and margins. No areas of necrosis or cavitation were found.

The peribronchial lymph nodes were in a dark gray dense state similar to that of the nodes on the right.

The heart was enlarged laterally, the enlargement being due to hypertrophy of the right ventricle and to right-sided dilatation. The left ventricle, auricle, valves and vessels and the aorta were of normal appearance and the chambers were filled with currant jelly clot which was beginning to soften.

The liver, spleen, kidneys, pancreas, gastro-intestinal tract and other abdominal organs and structures were of normal appearance and position to superficial examination.

Microscopic Examination

Lung. The pleura was thickened by fibrillar and partly hyalinized connective tissue throughout, that of the left lung being covered by a layer of fibrin containing a few leukocytes. Sections from the right lung showed variable degrees of collapse of the vesicles in the relatively non-fibrosed portions. In the apex of the right lung some small vessels showed thrombosis and there was fresh hemorrhage, probably related to a rupture which caused the pneumothorax.

In the apices, with areas of emphysema, there were a number of rounded, laminated, hyaline nodules containing deposits of fine, granular dustlike particles. These nodules were in part separate but also occurred in groups. In addition there were deposits of black dust in a perivascular distribution. The alveoli in some areas showed an accumulation of edema fluid, and in this also there were scattered fine dust particles.

Sections from the lower parts of the lungs revealed a condition similar to that found in the apices, but greatly aggravated. Numerous single and grouped hyaline, laminated nodules, in which occurred deposits of fine dust particles, occupied a large portion of the lung space. In the periphery of these nodules, and between them when they were grouped, there was proliferation of young fibrous tissue. There was variable emphysema and collapse and in many open alveoli edema fluid discol-

ored by fine dust particles was encountered. In some sections practically all of the lung tissue was obliterated by masses of hyaline nodules, with intervening cellular fibrous tissue.

The bronchi in the dense fibrous areas were in a state of partial to complete collapse and the mucosa of the large bronchi showed epithelial metaplasia.

Liver. In addition to central lobular congestion and associated deposit of brown, granular, iron-bearing pigment, there were deposits of fine granular, opaque dustlike material, located also about the central lobular venules and in the adjacent sinusoids. In addition, this material was found in the connective tissue about the collecting veins. It was accumulated particularly in Kupffer cells in the central lobular, swollen zone, giving a grayish cast to these cells under low magnification. It also occurred in hyaline and fibrillar strands of apparently necrotic material in the central lobular zone, among which were scattered nuclear particles. Where focal deposits were heaviest and liver cells were degenerated or necrotic, there were a few leukocytes and also a few large spindle-shaped fibrous tissue cells. While there was no tissue change shown in the portal vein and bile duct, high magnification revealed some fine granular deposit in the surrounding connective tissues.

The picture given by the liver was that of foreign dustlike material which had been taken out of circulation by the Kupffer cells of the central zone and which, where deposited, had led to degeneration and necrosis of tissue, with hyalinization and early fibrous tissue proliferation. Since practically every lobule showed some deposit, the volume appeared to be considerable with a material loss of liver substance.

Spleen. The capsule was not materially altered. The walls of the arterioles of the malpighian corpuscles were usually, though not invariably, thickened and hyaline. The fibrous trabeculae were prominent and the walls of the vessels within them also showed hyaline thickening. The sinuses were generally full of blood and their walls were prominent and cellular, with the endothelial cells conspicuous. Within the walls of the sinuses, and especially in the endothelial cells, there was a deposit of fine, opaque, dustlike granules, so small that examination with the 16 mm. objective and the 10x ocular did not reveal them. Like material was also deposited in broad zones or in more or less rounded collections within the trabeculae, especially in the periphery of these structures, and within some of the malpighian bodies, here lying along the arteriole as a rule. In the trabeculae and malpighian bodies this material lay within more or less hyalinized tissue, which was neither especially rounded nor laminated, although in some instances there

was a suggestion of nodularity. Within this hyaline matrix were numerous, large, ovoid or spindle-shaped cells, more numerous at the periphery and fewer at the center of the malpighian body. Scattered about were nuclear fragments from necrotic cells. These also were more numerous in the periphery of the body.

The picture as a whole is that of deposit of very fine, opaque granular dust, in the endothelial cells of the sinuses and in nodular or band-like collections, stimulating connective tissue formation, hyalinization and even inducing necrosis, within malpighian bodies and the peripheral zone of the trabeculae.

Kidneys. Cursory examination of the kidneys showed no particular abnormality save congestion and cellular degeneration, attributable to postmortem autolysis. Closer study, however, revealed the presence of very fine opaque granules scattered in the endothelial cells of the capillaries of the glomeruli. There was no apparent change associated with their presence, unless there may have been some hyalinization of the basement membrane.

Peribronchial Lymph Nodes. The lymphoid tissue of the nodes adjacent to the large bronchi was, to a large extent, replaced by masses of hyaline nodules of various sizes, containing fine deposits of particulate matter. The centers of some of the larger nodules were calcified.

In none of the organs was there found any evidence of tuberculosis, and no acid-fast bacilli could be found in appropriately stained sections of liver and spleen. The Prussian blue test failed to demonstrate iron in the particles in any of the tissues.

The diagnosis at autopsy was: Pulmonary silicosis; acute pleurisy, left; pneumothorax, right; hypertrophy and dilatation of right heart. After microscopic examination, to this diagnosis the following was added: silicosis of spleen, liver, kidneys and peribronchial lymph nodes. Because of conditions surrounding the autopsy and since there appeared to be no abnormality of any organ except lungs and heart, only blocks of other viscera were taken for microscopic study.

Both grossly and histologically the condition of the lungs was typically that of silicosis and the deposits in the spleen and liver appeared to be of the same material. However, since the natural occurrence of silicosis outside the pulmonary area had not been observed, it was important that the material concerned should be identified.

Microchemical methods of analysis* were devised by F. B. Culp, of the Chemistry Department, since the tissue taken from the liver,

* Mr. Culp proposes to publish his methods of these examinations in a separate article.

spleen and kidney was only that which was intended for histologic study. In this chemical examination the lung was found to contain 1.6 mg. of silica to each gram of wet tissue and 11.15 mg. of silica to each gram of dry tissue. By referring to the chemical examination for silica in lungs in health and disease by McNally,[2] this will be found to be a higher content of silica than was found in all but two of his eight workers in dusty atmospheres, and significantly greater than his findings in lungs of persons not so exposed. Control tests on three non-diseased lungs, selected to have had a minimum of dust exposure, gave 0.050, 0.019 and 0.031 mg. of silica to each gram of wet tissue and 0.50, 0.13 and 0.18 mg. to each gram of dry tissue, respectively.

The same analysis applied to liver tissue in the present case revealed 0.10 mg. of silica to each gram of wet tissue and 0.70 mg. to each gram of dry tissue, while control tests on two livers having no discoverable similar deposits disclosed 0.015 and 0.005 mg. of silica per gram of wet tissue and 0.08 and 0.02 mg. per gram of dry tissue, respectively.

In the spleen the chemical examination revealed 0.2 mg. of silica per gram of wet tissue and 1.40 mg. to each gram of dry tissue, as compared to 0.078 and 0.033 mg. per gram of wet tissue and 0.46 and 0.23 mg. per gram of dry tissue, respectively, in two normal spleens.

The kidney yielded 0.02 mg. of silica per gram of wet tissue and 0.14 mg. per gram of dry tissue, as compared to 0.013, 0.008 and 0.01 mg. per gram of wet tissue and 0.09, 0.04 and 0.05 mg. of silica per gram of dry tissue, respectively, in three normal kidneys.

As a further control, the formaldehyde solution in which the tissue was fixed contained 0.001 mg. of silica per cc. of fluid, as did also freshly made formaldehyde fixing fluid.

It is therefore seen that the content of silica of the tissues concerned was significantly higher in lung, liver and spleen than in normal controls and even in the kidney was higher than in the three controls used.

Discussion

In spite of the difficulties attending positive identification of the dustlike particles in the spleen and liver as silica, there can be no question that it is the same material as seen in the lungs and, further, that there is actual and material damage to spleen and liver tissue by it.

It is interesting to note that the location of deposits in the liver is quite different from that found by Gardner and Cummings [1] in experimental silicosis of the liver, that being periportal in distribution and leading to a portal type of cirrhosis, while here the deposit is of central lobular location, associated prominently with Kupffer cells in the sinusoids and with degeneration and necrosis of liver cells and early fibrosis.

The material is not iron-bearing, it is morphologically like the dust deposits in the lung, the tissues containing it were shown to hold a significant amount of silica by chemical examination, the tissues damaged were in direct relation to the dustlike deposits, and neither the distribution, the tissue change, nor bacteriologic staining indicates that there is associated tuberculosis.

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DESCRIPTION OF PLATE

PLATE 56

FIG. 1. Hyaline fibrosis of lung. Silica particles too small to be seen at this magnification. × 67.

FIG. 2. Hyaline fibrous nodule in spleen, with particulate deposit. × 226.

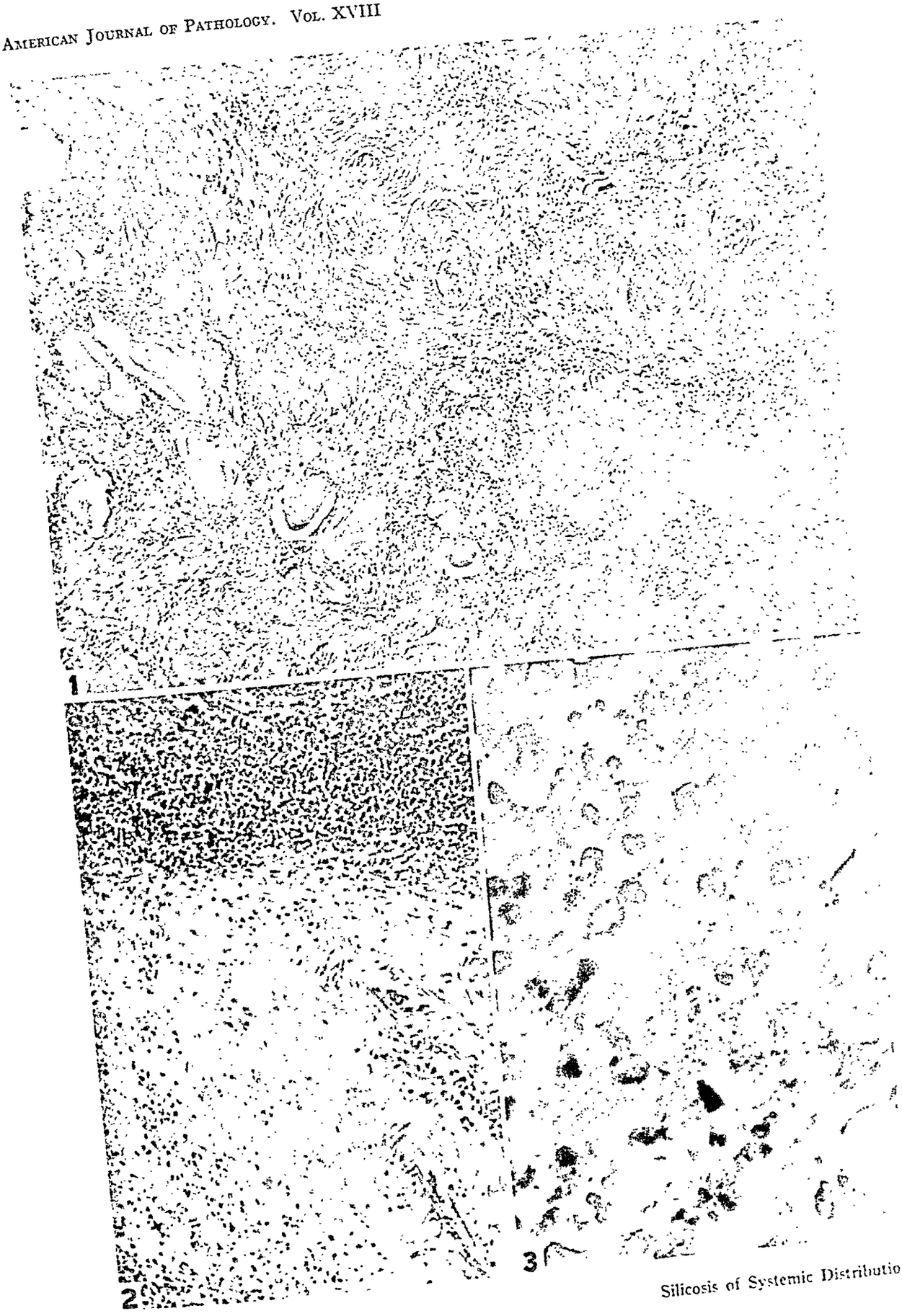
FIG. 3. Deposit of particulate material with cellular degeneration in the liver in silicosis. × 744.

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liver in silli-



Silicosis of Systemic Distribution

EXPERIMENTAL ALLERGIC FOCAL NECROSIS OF THE LIVER*

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Although focal necrosis of the liver is common in infectious and toxic diseases, its nature and etiology are yet not clearly understood. Some believe that this lesion is caused by mechanical ischemia which is secondary to thrombosis of nearby veins and sinusoids, while others think that it is due to the direct action of toxic substances on the hepatic parenchyma without thrombus formation. In the present investigation we have attempted to determine whether thrombosis or direct action of toxic substances, or both, are responsible for the development of focal necrosis of the liver by studying this lesion in hypersensitive animals.

MATERIALS AND METHODS

Most of the animals used in this work were rabbits weighing approximately 2000 gm., although a few guinea pigs were used to determine whether other species might show the same reaction. Injections of 1 cc. of 3 per cent, highly purified, crystalline egg-albumin were made into the radicles of the mesenteric veins, into the marginal ear veins, into the abdominal cavities and directly into the spleens of rabbits which had been previously made hypersensitive by repeated subcutaneous injections of the same protein. From 0.2 cc. to 0.5 cc. of the same protein was injected into the mesenteric veins of hypersensitive guinea pigs. Most of the animals survived this procedure and were sacrificed from 2 hours to several months afterwards. In the earlier part of the work some of them died on the operating table from generalized anaphylactic shock, but it was soon found that this could be prevented if the crystalline egg-albumin was injected very slowly (about 0.15 cc. per minute). A small wedge-shaped piece of liver was taken from each animal at the time of the first operation. After the animals had been sacrificed or had died, the livers, spleens, lungs, hearts and kidneys were examined grossly and microscopically. The tissues were fixed in Zenker's fluid or in a 2 per cent solution of formaldehyde. Sections were stained routinely with hematoxylin and eosin, or with Maximow's hematoxylin-eosin-azure and occasionally with scharlach R and with Mallory's phosphotungstic acid connective tissue stain.

* Presented at the Fortieth Annual Meeting of the American Association of Pathologists, Pittsburgh, Pa., March 22, 1940.
Received for publication, July 26, 1941.

Approximately 30 animals were given from two to nine injections of crystalline egg-albumin into the mesenteric veins at about 1-week intervals in an attempt to determine whether fibroplastic reactions could be produced in the livers. The animals were then sacrificed and their tissues were studied grossly and microscopically as before.

To determine the specificity of the reaction, one group of rabbits was sensitized with crystalline egg-albumin and later given an injection of horse serum into the mesenteric veins, while another group was sensitized with horse serum and later given an injection of crystalline egg-albumin into the mesenteric veins. All animals of both groups were sacrificed after 24 hours and their tissues were examined grossly and microscopically as above.

Observations

Grossly, the 24-hour lesions were yellowish gray, round, sharply demarcated areas, and measured from pin-point size to 1 mm. in diameter. They were present throughout the liver but were more numerous immediately beneath the capsule.

Microscopically the lesions were focal, either midzonal, peripheral, or almost central in the lobules. True central necrosis, however, was not seen. The lesions varied in size from very small ones, affecting but 20 or 25 cells, to large areas, involving almost the entire lobule. More than one lesion was occasionally present in a lobule and a single lesion sometimes extended to more than one lobule (Fig. 1). Definite foci of beginning coagulative necrosis of the parenchymal cells were seen as early as 8 hours after the injection of the shocking dose of crystalline egg-albumin. By this time, but not before, the sinusoids in the involved areas and many nearby portal veins were markedly congested with polymorphonuclear leukocytes (Fig. 2). Within from 18 to 24 hours most of the affected hepatic cells had lost their integrity; the cells had become homogeneous, granular, eosinophilic masses containing an occasional faded nucleus. The necrotic liver cells were sharply demarcated from the rest of the lobule, and many of the less damaged cells immediately adjacent to the edge of the lesion contained fat droplets (Fig. 3).

Hemorrhage into the necrotic areas was seldom seen, probably because the connective tissue framework of the lobule remained intact despite the massive necrosis of the parenchymal cells (Fig. 4). An occasional thrombus was seen in a portal triad vein near the intralobular lesions, but it should be emphasized that these thrombi were relatively rare in comparison with the large amount of necrotic tissue which was present. After 4 or 5 days many of the necrotic cells and

leukocytes had disintegrated or were in the process of disintegration. After 10 days the necrotic areas had usually disappeared completely, their places having been taken by regenerated liver cells.

Although some animals received as many as nine injections of the antigen into the mesenteric vein, very little increase in the hepatic fibrous tissue was found. Occasionally, however, definite evidence of the experimental production of fibrosis was obtained in livers with normal parenchyma, as shown by biopsy before the injections were begun. Such lesions were located beneath the capsule. The fibrosis was chiefly in the portal areas, but it was also present within the lobule, disrupting the normal lobular architecture (Fig. 5).

None of the control animals that were given injections of crystalline egg-albumin into the mesenteric veins after they were hypersensitized with horse serum showed hepatic changes. Conversely, none of the animals that received horse serum into the radicles of the mesenteric vein after being hypersensitized to crystalline egg-albumin showed liver changes.

Although most of the shocking doses of antigen were injected into the mesenteric veins to insure a constant amount of antigen reaching the liver, massive areas of liver necrosis were present following intra-abdominal, intrasplenic, and peripheral intravenous (marginal ear vein) injections of antigen. The latter route is especially interesting because the antigen had to pass the pulmonary capillary bed before reaching the systemic circulation. It is true that a high percentage of hypersensitive rabbits will die of acute anaphylactic shock following the marginal ear vein injection of the specific antigen if the latter antigen is injected rapidly, but these accidents can usually be avoided if the antigen is injected slowly (0.15 cc. per minute).

The hypersensitive guinea pigs showed massive areas of liver necrosis similar in every respect to the hepatic necrosis observed in rabbits following injection of the specific antigen into the mesenteric vein. This was interesting in view of the general assumption that the lung is the only important site of allergic manifestations in this animal.

No changes were observed in the other tissues (myocardium, lung, kidney, spleen) in any of the animals used in this study.

Discussion

It has been suggested (Mallory,[1] Pearce,[2] Karsner and Aub [3]) that focal necrosis of the liver may be secondary to thrombosis of venules and intralobular sinusoids with a resultant pure mechanical ischemia, and this lesion is occasionally seen at necropsy following propagating thromboses of the portal system. The rarity of this finding in focal

necrosis in human necropsy material indicates that this is not the usual way that this condition is produced. Loeb and Meyers,[4] for example, produced hepatic focal necrosis by the direct action of ether on the parenchymal cells, showing that toxic chemicals alone could produce it without the production of thrombosis. Chemical poisons, however, also are rare in human cases in which focal necrosis of the liver is found. It is possible that liver necroses in cases of idiosyncrasies to drugs and proteins, and sensitizations to bacteria and their products, are secondary to an antigen-antibody union on or within the hepatic cells. Opie[5] has shown that when a foreign protein and its specific antibody are mixed *in vitro,* a precipitate forms which produces inflammation and necrosis when injected into the skin of the normal animal. In passively sensitized animals, necrotic skin lesions form at the site of injection of the specific antigen, but if the antibodies are first precipitated out of the immune serum by treatment with antigen and the supernatant serum is then injected into the normal animal, no local lesion is produced by subsequent intracutaneous injection of antigen (Culbertson[6]). From this it is concluded that the antigen-antibody union is necessary for the production of necrosis. If the necrosis of the liver produced in our experiments (which is similar to that seen in human necropsy material) is secondary to a similar antigen-antibody reaction, one would suspect that this reaction would take place within the liver lobules where the relatively slow circulation and the large surface area allow the antigen the best opportunity to make contact with the antibody on or within the liver cells. Because of the location of the necroses in this area and the relative absence of vascular thrombi, it would seem that the focal hepatic necroses here observed are secondary to a local antigen-antibody reaction.

Rich and Follis,[7] working with normal avascular and vascularized rabbit corneas, concluded that the primary site of action in the Arthus type of hypersensitivity is the vascular endothelium and that tissue death is the result of impairment of nutrition due to vascular damage. We have seen no evidence of sinusoidal endothelial damage in this type of reaction in the liver but realize, of course, that the endothelial cells may be damaged without presenting any morphological changes. However, the absence of edema and hemorrhage from the intralobular lesions is evidence against the presence of endothelial damage.

Summary

1. Massive areas of coagulative necrosis of liver parenchyma were produced by the injection of crystalline egg-albumin into the peritoneal cavity, spleen, or into the mesenteric or marginal ear veins of rabbits previously sensitized to the same protein.

2. Similar hepatic lesions were produced in hypersensitive guinea pigs following the injection of crystalline egg-albumin into the mesenteric veins.

3. Portal fibrosis was occasionally seen following multiple injections into the mesenteric veins of hypersensitive rabbits, but this condition was difficult to produce.

4. Because it was so rarely present in comparison to the large number and large size of the lesions, vascular thrombosis is considered secondary to the massive necrosis of the hepatic cells.

5. No changes in the vascular endothelium and no hemorrhage or edema in the intralobular lesions were observed.

6. Congestion of portal veins and sinusoids by large numbers of polymorphonuclear leukocytes was not observed before areas of beginning coagulative necrosis were present.

7. It would seem, therefore, that the local union of antigen and antibody is responsible for the focal necrosis, and that the manifestations of the response; *i.e.*, coagulative necrosis, polymorphonuclear leukocytic response, and occasional thrombosis of adjacent veins and sinusoids, are all secondary to this antigen-antibody union.

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DESCRIPTION OF PLATES

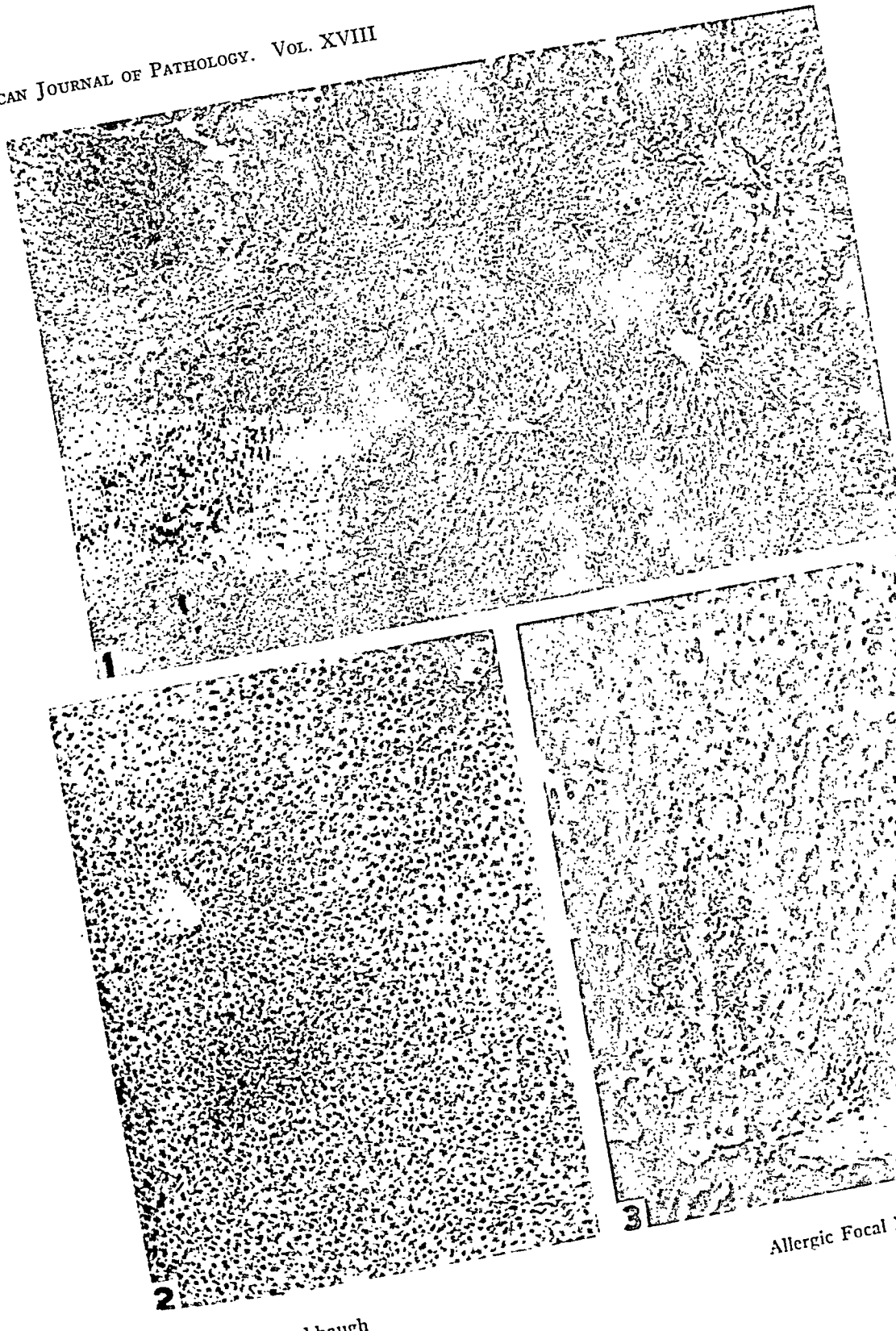
PLATE 57

FIG. 1. Photomicrograph of a typical liver lesion 24 hours after the injection of 3 per cent crystalline egg-albumin into the mesenteric vein of a hypersensitive rabbit. Massive areas of focal coagulative necrosis and polymorphonuclear leukocytic infiltration (dark areas in photograph) can be seen. × 59.

FIG. 2. A very early lesion (8 hours after the injection of antigen into the mesenteric vein). Beginning focal accumulation of polymorphonuclear leukocytes is shown in the lower left quadrant of the photomicrograph. Thrombosis is absent in nearby veins. × 95.

FIG. 3. A 24-hour lesion showing a typical, well demarcated area of coagulative necrosis of hepatic cells and polymorphonuclear leukocytic infiltration into the affected area. There is an absence of thrombosis in the nearby portal vein as well as of hemorrhage in the lesion. × 174.

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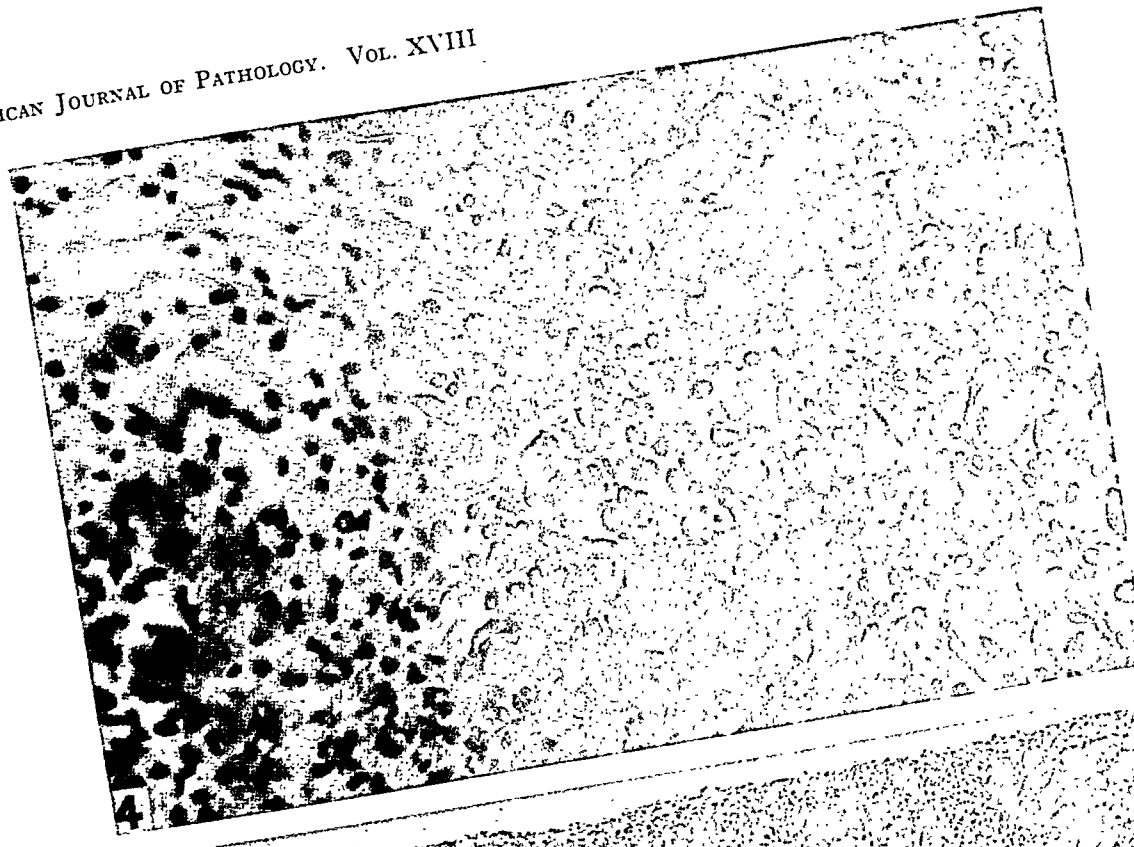
Hartley and Lushbaugh

PLATE 58

FIG. 4. Photomicrograph of a 3-day lesion. Relatively normal liver cells are present in the right upper quadrant while the hepatic cells in the left lower quadrant have undergone coagulative necrosis. The connective tissue framework of the involved area is intact and hemorrhage is absent. There is also a beginning infiltration of inflammatory cells into the affected area and an appearance of fat droplets in some of the less involved cells adjacent to the lesion. × 329.

FIG. 5. An area of sub-capsular portal fibrosis. This rabbit had had 5 mesenteric intravenous injections, each of 1 cc. of 3 per cent crystalline egg-albumin, over a period of about 8 weeks. × 66.

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Allergic Focal Necrosis of the Liver

Hartley and Lushbaugh

PROTEOLYTIC DIGESTION OF RED AND WHITE BLOOD CORPUSCLES IN THE SPLEEN*

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Enlargement of the spleen occurring in response to infection, usually designated as acute splenic tumor, is in some instances associated with injury or destruction of red blood corpuscles, and phagocytosis of red cells is conspicuous in the pulp of the spleen. These changes are well illustrated by the acute splenic tumor of typhoid fever; the spleen is soft and may be diffluent.

When washed erythrocytes of the pigeon were injected by Addison[1] into the blood stream of rabbits, phagocytosis of these cells by mononuclear phagocytes proceeded very actively in the sinuses of the pulp of the spleen and phagocytes were largest about 16 hours after injection. Perfusion of the spleen facilitated the histological study of the changes in the pulp.

Wehrle,[2] in this laboratory, studied the fate of the red and white blood corpuscles of the rabbit introduced through an ear vein into the circulating blood of animals of the same species. There was active phagocytosis, and intracellular digestion of both erythrocytes and granulocytes was seen in the splenic cords and was somewhat less in the sinuses. Perfusion of the spleen washed the red corpuscles and lymphocytes from both cords and sinuses, but, as actual count showed, left mononuclear phagocytes and granulocytes almost undisturbed. Phagocytosis of erythrocytes and of granulocytes, determined by counts of the number of mononuclear cells that had ingested these cells, increased, reached a maximum between 16 and 18 hours after injection and then diminished.

Experiments have been undertaken to determine if there is increase of proteolytic activity of splenic tissue corresponding with the phagocytosis of red and white blood cells that occurs following their introduction into the blood stream.

METHODS

Young rabbits weighing usually from 1700 to 2300 gm. were used. Blood corpuscles, obtained by intracardiac puncture with aseptic precautions, were washed three times with normal salt solution and then brought to the original volume of the blood. Twenty cc. of this suspension of red and white cells were injected into the lateral ear vein of

* Received for publication, July 31, 1941.

each rabbit and the animals were killed at different intervals after the administration of the cells. The spleen was immediately perfused with salt solution passed through a cannula inserted into the aorta at the level of the celiac axis. After ligation of all arteries except the splenic, the perfusion fluid escaped from the splenic vein. Perfusion was continued for 10 to 20 minutes until the color of the spleen changed from purple to orange-pink. The method was, in general, the same as that used by Wehrle,[2] but the spleen was perfused with a pressure of 60 cm. of water instead of 120 cm.

A weighed quantity of the perfused spleen ground to a pulp was incubated with pure casein at 37° C. and proteolytic digestion was measured by increase in nonprotein nitrogen. The nonprotein nitrogen content of samples of the mixture removed at different intervals after the beginning of incubation was determined. Protein was precipitated with trichloracetic acid and removed by filtration. The nitrogen content of the filtrate was determined by micro-Kjeldahl analysis. The initial nonprotein nitrogen present in the mixture of spleen and casein was determined in a sample removed as soon as the mixture was prepared, and when this figure was subtracted from that obtained from samples removed at different intervals after incubation at 37° C., the remainder was the measure of the digestion that had occurred. The nonprotein nitrogen in the mixture before incubation in all instances varied from 4 to 6 mg. per gram.

Digestion occurred in the presence of physiological salt solution or of Sorenson's phosphate buffer at pH 6.3, 6.7, 7.0, or 7.4, the hydrogen ion concentration being checked colorimetrically with bromthymol blue. Toluene was used as a bacteriostatic agent. The mixture was made up in the following proportions: 1 gm. spleen, 0.5 gm. casein and 50 cc. of buffer solution, the actual volume being adjusted to the quantity of spleen that was available. Preliminary experiments had shown that 1 gm. of spleen incubated alone and thus allowed to undergo autolysis gave values varying from 8 to 13 mg. of nonprotein nitrogen. It is convenient to express the results of digestion in milligrams of nonprotein nitrogen obtained by the action of 1 gm. of spleen. Casein was incubated alone at hydrogen ion concentration varying from pH 6.3 to 7.4 and there was no increase of nonprotein nitrogen during 30 hours of incubation. The casein that was used contained 13 per cent nitrogen, 0.5 gm. containing 68 mg. of nitrogen. It may be assumed that figures for nonprotein nitrogen in excess of 8 to 13 mg. per 1 gm. of spleen are the result of digestion of the casein present in the mixture.

All animals were autopsied and routine microscopical sections made from lung, liver and kidney to exclude the presence of disease, and no pathological change was found in any of the animals. Microscopical

sections of the perfused spleens were examined to determine the activity of phagocytosis. The results of these observations were in accord with those of Wehrle,[2] but actual counts of phagocytes were not made.

In preliminary experiments proteolysis was measured after 24 hours of digestion and the results are shown in Table I. The figure representing nonprotein nitrogen of the mixture before incubation has been subtracted from that obtained after digestion.

TABLE I

Proteolysis Produced by Normal Spleen and by Spleens of Animals That Had Received Blood Corpuscles

Rabbit no.	Blood corpuscles injected into vein	pH	Increase of nonprotein nitrogen after 24 hours at 37° C.
7	None	No buffer	9 mg. N. P. N. per gm. of spleen
		pH 6.3	26 mg. N. P. N. per gm. of spleen
		pH 6.3	21 mg. N. P. N. per gm. of spleen
		pH 6.7	21 mg. N. P. N. per gm. of spleen
8	None	pH 6.7	21 mg. N. P. N. per gm. of spleen
		pH 7.4	4 mg. N. P. N. per gm. of spleen
9	Corpuscles from 20 cc. of blood given 17 hours before animal was killed	pH 6.7	51 mg. N. P. N. per gm. of spleen
10	Corpuscles from 20 cc. of blood given 17 hours before animal was killed	pH 6.7	48 mg. N. P. N. per gm. of spleen
11	Corpuscles from 20 cc. of blood given 17 hours before animal was killed	pH 6.7	53 mg. N. P. N. per gm. of spleen
22	Same but with no perfusion of spleen	pH 6.7	17 mg. N. P. N. per gm. of spleen

When no buffer was used to maintain the original hydrogen ion concentration of the mixture, digestion was inhibited, presumably by the accumulation of products of digestion. With pH of 6.3 and of 6.7, digestion during 24 hours produced by normal spleen was active, but with greater alkalinity, namely, with pH 7.4, scant digestion occurred. The proteolytic activity of splenic tissue 17 hours after the introduction of blood corpuscles into the circulating blood was much greater than that of normal spleen.

Proteolysis caused by spleen with no perfusion was found to be much less than that caused by perfused splenic tissue. In Table II, representing several similar observations, digestion at pH 6.7 caused by tissues from a spleen with no perfusion, removed from an animal that had received blood corpuscles 17 hours before death, is compared with that caused by a perfused spleen from a similarly treated animal. Digestion is evidently inhibited by the presence of blood serum within the spleen and proceeds more actively when this is removed by perfusion.

The introduction of granulocytes into the circulating blood, their accumulation in the spleen, and phagocytosis by mononuclear phagocytes within the pulp and sinuses suggested the possibility that the

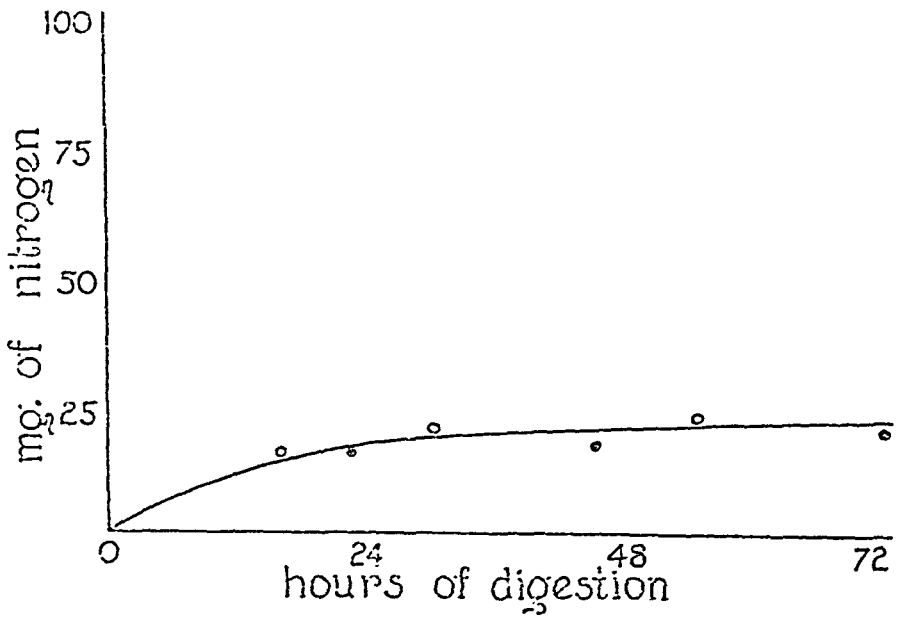
weak leukoprotease of the granulocytes of rabbits might have a part in the proteolysis demonstrated by the foregoing experiments, but the scant proteolysis in the presence of an alkaline medium, namely, pH 7.4 (Table I), showed that proteolysis by leukoprotease was an insignificant factor in the increased proteolysis caused by splenic tissue following intravenous injection of blood corpuscles. This increased digestion occurred under conditions that favored the activity of the

TABLE II

Spleen with no perfusion (no. 22)		Spleen with perfusion (no. 28)	
Hours of digestion	Increase of nonprotein nitrogen in mg.	Hours of digestion	Increase of nonprotein nitrogen in mg.
24	17	24	50
50	19	48	77
72	19	72	82
Nonprotein nitrogen before digestion was 4 mg.		Nonprotein nitrogen before digestion was 4 mg.	

proteolytic enzyme found in histiocytes that have assumed the function of macrophages.[3]

In subsequent experiments the proteolytic action of splenic tissue on casein at different intervals after intravenous injection of blood



TEXT-FIGURE 1. A composite graph showing digestion produced at pH 6.7 by 1 gm. of splenic tissue from two normal animals.

corpuscles was measured after increasing periods of digestion up to 72 hours. Digestion was measured with hydrogen ion concentration adjusted by buffer solutions to pH 6.7, 7.0 and 7.4, and was found to be most active at pH 6.7. No digestion occurred during 30 hours at pH 7.4.

Text-figure 1 is a composite curve prepared to show the results of di-

gestion at pH 6.7 caused by splenic tissue of two normal animals. There has been no increase of proteolysis in the period after 30 hours of incubation, and the non-coagulable nitrogen has risen to a maximum of less than 25 mg. of nitrogen.

Text-Figures 2 to 5 show, after different periods, the activity of digestion caused by perfused splenic tissue removed 6, 17, 24 and 48 hours after injection of blood corpuscles into the circulating blood. Within 6 hours after injection of corpuscles the activity of proteolysis has increased greatly above normal and has maintained this level for at least 24 hours. After 48 hours the curve of digestion has returned to normal.

TABLE III

Duration of digestion	pH	Increase of nonprotein nitrogen*
24 hours	6.7	28 mg.
72 hours	6.7	36 mg.

* Nonprotein nitrogen before digestion was 4 mg.

TABLE IV

Duration of digestion	pH	Increase of nonprotein nitrogen*
24 hours	6.7	50 mg.
48 hours	6.7	71 mg.
72 hours	6.7	71 mg.
24 hours	7.0	15 mg.
48 hours	7.0	24 mg.
72 hours	7.0	47 mg.

* Nonprotein nitrogen before digestion was 4 mg.

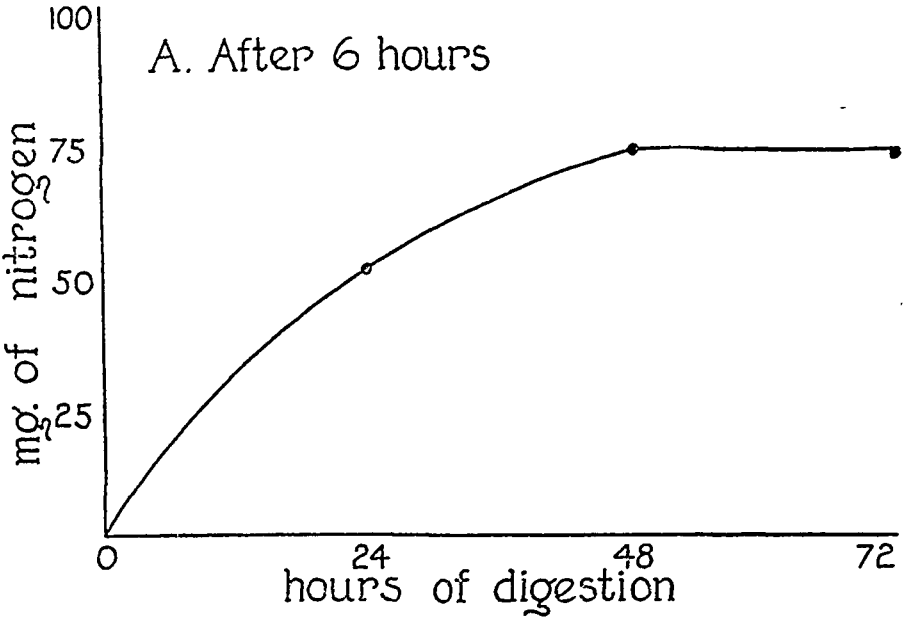
Text-Figure 6 shows the activity of digestion at increasing intervals after intravenous injection of blood corpuscles under conditions that were found favorable for proteolysis, that is, at pH 6.7, and after a uniform time interval of 24 hours of digestion. It is noteworthy that the curve rises promptly and maintains an almost uniform level from 6 to 24 hours, being highest at 17 hours, and then falls to a normal level 48 hours after injection of blood corpuscles.

Two experiments were performed to determine the effect of repeated injection of blood corpuscles on the proteolytic activity of splenic tissue.

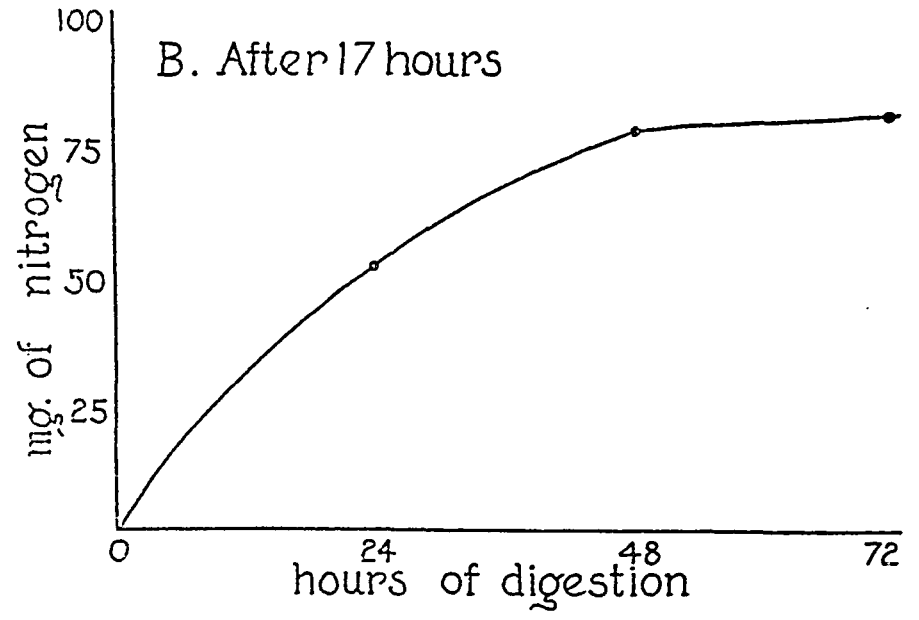
A rabbit (no. 26), weighing 2000 gm., received on 3 successive days washed blood corpuscles from 20 cc. of blood. When the animal was killed 12 hours after the last injection, the spleen measured 42 by 9 by 5 mm. Digestion by the perfused spleen in the presence of casein at pH 6.7 is shown in Table III.

A rabbit weighing 2700 gm. received washed blood corpuscles from 20 cc. of blood on 4 successive days and was killed 24 hours after the last injection. The spleen measured 55 by 14 by 8 mm. The progress of digestion with casein caused by the perfused spleen is shown in Table IV.

Digestion by splenic tissue after repeated intravenous injections of blood corpuscles has been more active than that produced by normal splenic tissue, but proteolytic activity of the same quantity of tissue has not increased above a maximum represented by that following a



TEXT-FIGURE 2. Graph showing the activity of proteolytic digestion at pH 6.7 caused by 1 gm. of splenic tissue (A) 6 hours after intravenous injection of blood corpuscles.

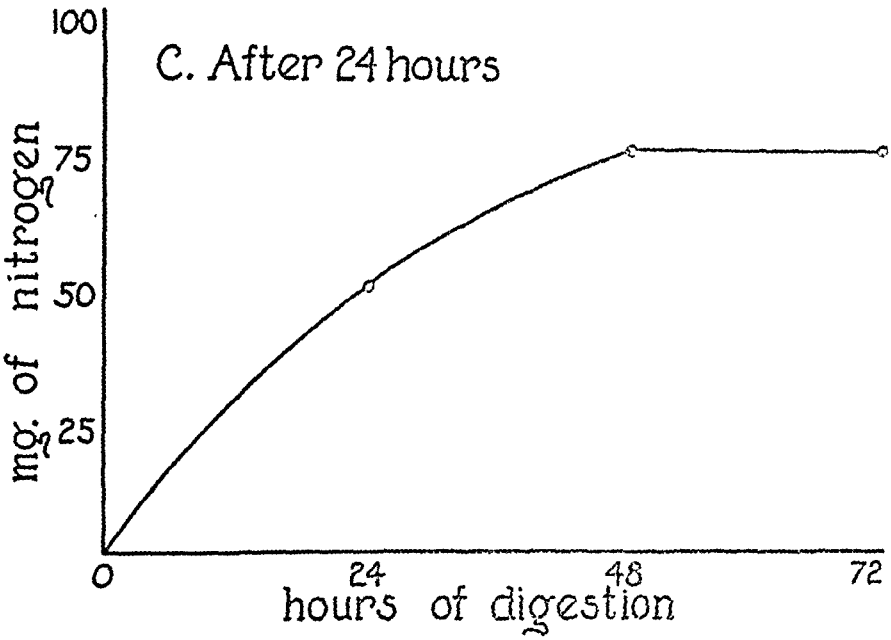


TEXT-FIGURE 3. As in Text-Figure 2: (B) 17 hours after injection.

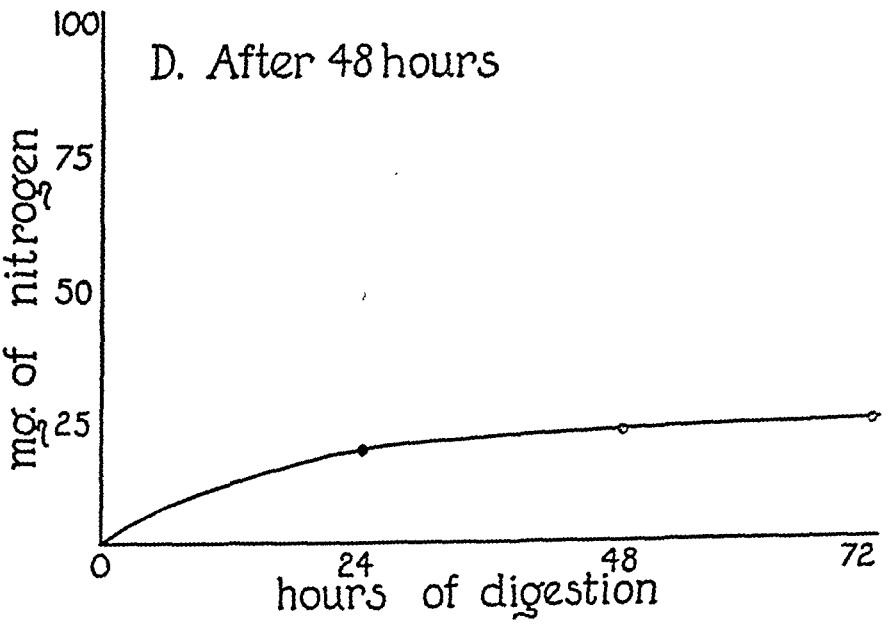
single injection of blood corpuscles (Table II). It is noteworthy that the volume of the spleen in the second experiment had increased to considerably above normal.

Discussion

A single injection of washed corpuscles, Wehrle [2] found, increased the size of the spleen from a normal average of 43 by 6 by 2 mm. to 53 by 9.4 by 3 mm. Rous and Oliver [4] injected 10 cc. of citrated whole

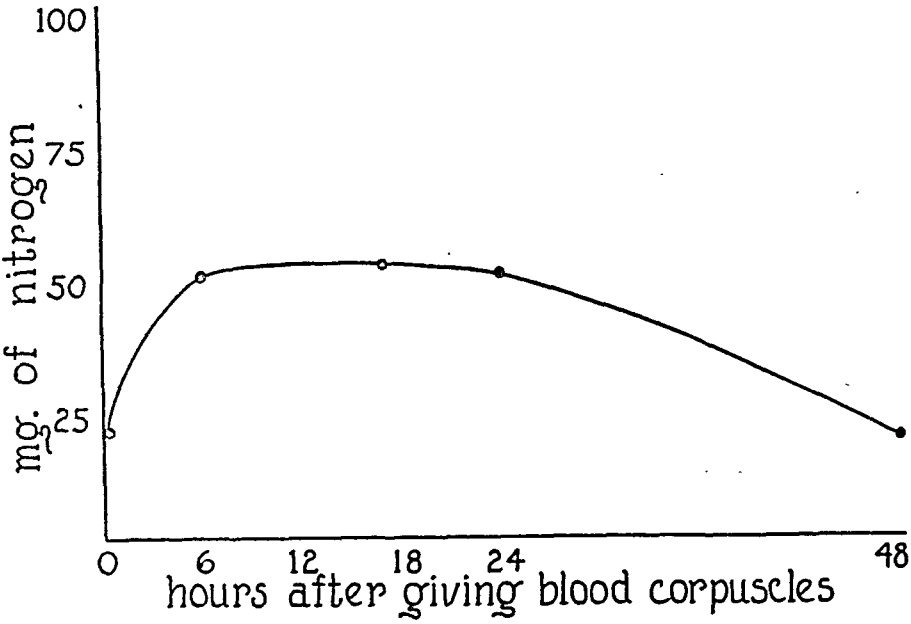


Text-Figure 4. As in Text-Figure 2: (C) 24 hours after injection.



Text-Figure 5. As in Text-Figure 2: (D) 48 hours after injection.

blood into the circulation of rabbits 6 days each week for several months. After 3 or 4 weeks the spleen became much enlarged and was made turgid by phagocytes crowded with red cells and cellular débris.



TEXT-FIGURE 6. Graph showing the activity of proteolytic digestion during 24 hours at pH 6.7, caused by 1 gm. of splenic tissue removed from rabbits at different intervals after introduction of an excess of blood corpuscles into the circulating blood.

After months of this plethora the spleen was diminished in size and the sinuses contained masses of hemosiderin.

The changes in the spleen found in our experiments are analogous to those of the red type of acute splenic tumor described by Evans [5] and distinguished by him from a gray type of acute splenic tumor, the former being associated with typhoid fever and related infections and the latter, he believed, with pneumococcal, staphylococcal, streptococcal and other infections.

It is noteworthy that phagocytosis and intracellular digestion of granulocytes as well as of erythrocytes occur within the pulp cords and sinuses of the spleen of normal rabbits (Wehrle [2]). Normal splenic tissue contains an enzyme capable of splitting protein in the presence of an acid medium. Its activity can be measured only after blood is removed from the spleen by perfusion. Parallel with the increase of phagocytosis of washed red and white corpuscles following the introduction of an excess of these cells into the circulating blood, there is increased proteolytic activity of splenic tissue, measured by its ability to bring about the digestion of casein. This increased proteolytic activity reaches a high level soon after injection of blood corpuscles, maintains this elevated level from 6 to 24 hours after injection of corpuscles and has disappeared 48 hours after the injection.

The enzyme that causes proteolysis in the spleen acts best in the presence of hydrogen ion concentration of pH 6.7 and is almost com-

pletely inhibited by an alkaline reaction of pH 7.4. In these characters it resembles the proteolytic enzyme that is present in the mononuclear phagocytes of an inflammatory exudate (Opie [3]) and in the macrophages that accumulate in lymph nodes adjacent to the site of inflammation. It is distinguishable from the leukoprotease of the granulocytes, which are ingested by the phagocytes of the spleen, by its failure to act in the presence of an alkaline medium.

Summary and Conclusions

Parallel with increased phagocytosis of red and white blood corpuscles in the spleen after injection of these cells into the circulating blood of rabbits, a measured quantity of splenic tissue causes increased proteolysis *in vitro*. Increased proteolysis is caused by a proteolytic enzyme present in the macrophages of the organ and measurable only after blood has been washed from the splenic tissue by perfusion.

This proteolytic activity is maintained at a high level from 6 to 24 hours following introduction of an excess of corpuscles into the blood and has returned to normal after 48 hours.

Parenteral digestion is a significant function of the spleen and proceeds with increased activity in some forms of "acute splenic tumor."

Note: We are indebted to Edward S. Howe and Frances W. Lovejoy for technical assistance in this investigation.

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SUBAORTIC STENOSIS*

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The less frequent cardiac malformations are always of pathologic interest. The example of subaortic stenosis encountered by us is especially interesting because a correct diagnosis was made during life.

REPORT OF CASE

History. R. H. K., a white male, 26 years old, entered the Multnomah County Hospital on March 23, 1935, complaining of cough, weakness and a swollen jaw. At the age of 10 years the patient had scarlet fever. Following this he learned that he had a "blow" in the heart and found he could not run as fast as his playmates. For the past 2 years he had not felt well, had tired very easily and had noted an increased pallor. Three months before admission sweats and chills developed, and for the last 3 to 4 months he had been coughing up about an ounce of sticky sputum a day. Following extraction of several carious teeth 1 month earlier, several small, painful, hemorrhagic spots developed at the tips of the fingers. There was no history of chest pain, nocturia or dependent edema. At the age of 16, after hard physical exertion, he was cyanotic for a few hours.

Physical Examination. Pulse, 110 per minute; respirations, 24; temperature, 101.0° F.; blood pressure 102/80. The important physical findings were as follows: The gums were swollen and bled very easily. There was no cyanosis. The heart was enlarged chiefly to the left. There was no lower sternal propulsion noted. A definite systolic thrill, detected over the second right interspace, was transmitted to the great vessels of the neck and down the left side of the sternum. The pulmonary second sound was not felt. A purely systolic murmur, most evident in the second right interspace, was transmitted to the vessels of the neck and down across the sternum to the left. The heart rhythm was regular. The aortic second sound had a clicking character. It was short, sharp and sounded close to the ear. The peripheral pulse was small and poorly sustained. The spleen was enlarged to palpation.

Laboratory Data. On entrance the following determinations were made: hemoglobin, 38.2 per cent; red blood cells, 2,490,000; color index, 0.72; white blood cells, 7,050; polymorphonuclear leukocytes, 61 per cent; lymphocytes, 21 per cent; staff cells, 12 per cent; sedimentation rate, 110 and 156 mm. (15 and 45 minutes). Evidence of a moderate degree of erythropoiesis was observed in the blood film. The Kolmer and Kahn reactions were negative. Urine: specific gravity, 1,010; yellow; acid, and cloudy with occasional hyaline and granular casts. Pus cells ranged from ++ to +++ and erythrocytes were listed as + in one of two examinations. Streptococci and gram-negative bacilli were found in the blood cultures but were not identified further. Interpretation of the electrocardiographic tracings was "questionable coronary disease."

Clinical Diagnosis. (Dr. Maurice F. Gourley.) Rheumatic aortic endocarditis, probably with considerable calcification; superimposed subacute bacterial endocarditis; subaortic stenosis.

* Received for publication, July 5, 1941.

After a progressive downward course, the patient died 42 days after hospitalization. The temperature varied from 100° to 103° F., occasionally rising to 104°, while the pulse ranged from 90 to 110 per min.

POSTMORTEM EXAMINATION

The postmortem examination was performed 15 hours after death. The body appeared slightly undernourished and pallor was evident. Aside from edema of the ankles there were no external changes. About 150 cc. of transudate were present in the peritoneal cavity. Approximately a liter of similar fluid filled the right pleural cavity while the left contained about 200 cc. The spleen weighed 540 gm. and contained a large, yellow, anemic infarct. A branch of the splenic artery supplying this portion of the spleen was occluded by a septic thrombotic embolus in which there were plates of lime salt like those in the aortic valve. The kidneys were swollen, soft and varied from yellow to bright red. Microscopically, they showed typical subacute proliferative glomerulonephritis with occasional partially infarcted glomeruli attributable to embolic occlusion. The other salient findings were encountered only in the heart.

Heart

Gross Examination. There were approximately 100 cc. of clear fluid in the pericardial sac. The heart had a diameter of 14.5 cm. and weighed 450 gm. The left ventricle, from the base to the apex, measured 10 cm. The thickness of the myocardium on the left and at the base was 2.2 cm., and 0.7 cm. at the apex. The chamber seemed relatively small in comparison to the dilated right cavity. The wall of the right ventricle was 0.7 cm. in thickness. One cm. inferior to the base of the aortic valve ring there was an annular, shelflike stenotic ring consisting of pale, grayish white, smooth, firm tissue covered everywhere by intact endocardium. This band was located 0.6 cm. caudal to the right posterior aortic valve cusp, but approximated and fused with the bases of the anterior and left posterior leaflets (*Fig.* 1). The ridgelike structure extended 0.6 cm. into the ventricular cavity and was not more than 2 mm. thick at any point. The endocardium between the posterior aortic valve cusps and the subaortic ring was smooth, glistening, opaque and slightly irregular. Here was found an endocardial pocket, or pseudo-valve, with an orifice measuring 0.6 by 0.2 cm. Its ostium lay close to, and opened toward, the base of the left posterior aortic valve cusp. Through it a probe could be passed towards the infra-aortic ring for a distance of 0.5 cm. The ring had a circumference of 2.5 cm. It extended well onto the ventricular surface of the aortic leaflet of the mitral valve but lacked 0.8 cm. of reaching the junction with its chordae tendineae. The anterior leaflet of the aortic valve was normal

save for slight fenestration along its free edge. The right posterior leaflet, however, was quite large, measuring 2.5 cm. in breadth at its commissural attachments and 1.5 cm. in height. The left posterior leaflet was 1.7 cm. in breadth. The large right posterior cusp was thickened and indurated. Adherent to the ventricular surface of the half nearest the left posterior leaflet was a firm, grayish yellow to pink nodular vegetation measuring 0.6 by 0.9 cm. Adherent to the aortic surface of this same cusp (Fig. 1) was a moderate amount of granular, pinkish yellow material. Similar excrescences were present on the ventricular aspect of the left posterior cusp, mainly involving the half of the valve near the commissure separating the two posterior leaflets. The aortic valve ring measured 6 cm. in circumference. Over the intima of the ascending aorta about the orifice of the left coronary artery there was a yellowish to grayish pink sessile elevation measuring 0.6 to 0.7 cm.; otherwise the inner surface of the aorta was smooth and glistening. Except for the extension of the subaortic annular ring onto the mitral, this structure was negative. The mitral valve ring measured 8.0 cm. in circumference. A few small focal areas of fibrosis were seen in the papillary muscles of the mitral valve. There were no associated anomalies other than a slight anatomical patency of the foramen ovale.

Microscopic Examination. The free border of the subaortic band consisted of hyalinized fibrous connective tissue which was largely covered by intact, flattened endothelium. At the junction of the ring with the myocardium there was a single focus of lymphocytes. The endothelium along the inner edge of the aortic valve and over the ventricular surface was replaced by a thin zone of polymorphonuclear leukocytes. Near the base of the valve the tissue was thicker, hyalinized, contained more polymorphonuclear leukocytes caught in fibrin, and showed areas of calcification. Bacterial stains revealed moderate numbers of gram-positive cocci in the central portion of the vegetation and along the free ventricular border of the valve. Sections of the myocardium proper showed a few minute foci of recent necrosis, unaccompanied by inflammation, and slight hypertrophy of the muscle cells. No structures that could be interpreted as active or healed Aschoff bodies were observed.

Comment

Judging from the slight degree of cardiac hypertrophy (450 gm.), maximum thickness of the left ventricular wall (2.2 cm.) and the slight dilatation of the left chambers, the subaortic annular stenotic ring and thickened aortic cusps failed to produce much obstruction or incompetence, yet formed a sufficient barrier to the passage of blood to evoke

the systolic thrill and murmur detected clinically. Another fact indicative of diminution in output was the small and poorly sustained peripheral pulse. Clinically, the clear-cut aortic second sound was interpreted as evidence that this valve was functioning properly. The diagnosis of subaortic stenosis was based upon the evidence of aortic obstruction with retention of the second aortic tone. The presence of a small endocardial "bird's nest" pocket between the aortic valve and the subaortic ring is an additional point against full competency of the valve. Such endocardial pockets are usually found in association with valvular insufficiency, and, as in this instance, open in the direction of the regurgitation. The fact that the nidus lay superior to the subaortic ring and was somewhat recessed makes it difficult to believe that it could have been responsible for the clinical observations or that it contributed appreciably to the changes observed at necropsy. Before the annular ring was sectioned at necropsy the right posterior aortic valve leaflet was found to be directly in line with the probable flow of blood coming from the ventricle. Inasmuch as the largest vegetation was present here, it further strengthens the view that trauma plays a decided etiologic rôle in acquired valvular affections. Clinically it was thought that the bacterial endocarditis was secondary to carious teeth. Both may have been responsible for the associated glomerulonephritis. This latter condition, together with the blood-stream infection, adequately explains the intense hypochromic anemia. The pulmonary edema was thought to be secondary to cardiac failure.

Discussion

The subject of subaortic stenosis has been thoroughly studied by Abbott [1] who, in 1927, was able to catalogue twenty-six instances. Enzer,[2] in 1927, described an anomalous subaortic bicuspid valve, located 3 cm. below the aortic valve. This anomalous valve had chordae tendineae attached to a papillary muscle. This is not a clear-cut instance of subaortic stenosis and is not included in the total list of cases. Sternberg,[3] in 1930, described two cases of this condition. One occurred in a woman, 27 years old, in whom heart disease had been clinically recognized since the age of 5. At the age of 19 years a diagnosis of aortic stenosis was made. The illness that led to her death was a septic sore throat. At necropsy a subaortic annular ring was found 6 to 7 mm. below the aortic valve ring. This fibrous band was only 2 mm. in height and appeared smooth and white. The ventricles and auricles were slightly dilated; no other congenital or acquired lesions were present. The second case was that of a man, 77 years old, who had arteriosclerosis, pulmonary emphysema and, later, obstructive icterus. In this heart a small, irregular, fibrosed band extended from the lower part of

the membranous interventricular septum onto the aortic leaflet of the mitral valve. The aortic valve showed extensive calcification. Sergent, Launay and Imbert,[4] in 1932, reported an example of congenital subaortic stenosis occurring in a male, 21 years old, which was complicated by an acute aortic bacterial endocarditis. In this case the subaortic ridge rose 1 cm. above the level of the interventricular wall.

A recent report,[5] of a boy, aged 14 years, with congenital subaortic stenosis and narrowing of the brachiocephalic venous trunks, is a clinical study and makes no factual mention of subaortic stenosis. This instance consequently is not included in the present review.

Dormanns,[6] in 1939, reported an instance of subaortic stenosis in a male adolescent, 16 years and 9 months of age. This person had had measles as a child. On entering school a systolic heart murmur was discovered accidentally. Subsequent examinations revealed this murmur to have become more intense and audible over the entire cardiac area. Nevertheless he was active in athletics and had no apparent difficulty in various competitive sports. One morning, without significant previous exertion, he suddenly collapsed and died within 2 hours. Autopsy, performed 72 hours after death, revealed a heart weighing 375 gm., which had an anomalous subaortic ring and thickened aortic valve leaflets. The collar-shaped subaortic ring lay 7 mm. below the aortic valve ring and was wedge-shaped in cross section, measuring 3 mm. in width at its base and 3 mm. in height. It was located 21 mm. below the upper border of the aortic valve and 8 cm. from the apex of the left ventricle. The aortic valve ring was 5.5 cm. in circumference and all three of its leaflets were thick, whitish and opaque. The left ventricle was enlarged and dilated. The anomalous ring was formed of fibrous connective tissue lying beneath an intact endocardium. There was no significant cellular infiltration. In the aortic leaflets an increase of fibrous connective tissue was apparent as well as considerable myxomatous tissue beneath the endocardium over the free edge of the valve. Here, as in the stenotic ring, significant cellular infiltration was absent. The myocardium showed no changes. Stating that the examination revealed no evidence of fetal or post-fetal endocarditis, Dormanns explained the subaortic stenosis and the changes in the aortic valve as due to a complicated arrest of development. No other cardiac abnormalities were found.

We feel that our case is a true example of subaortic stenosis, bringing the apparent total to thirty-one.

As to the pathogenesis of subaortic stenosis we can do no better than to quote Abbott:[1] "Two groups are to be differentiated, *(a)* those in which the lesion is associated with other anomalies, in which case a true arrest of development with survival of the bulbus cordis in the aortic

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vestibule has occurred, and (*b*) a subaortic stenosis of inflammatory origin due to an infective process setting in [in] early postnatal or late prenatal life." While not susceptible of proof, the appearance of cardiac symptoms following scarlet fever at the age of 10 years, together with the lack of associated cardiac anomalies or evidences of arrested development, are points much in favor of an acquired lesion due to inflammation. Abbott [7] further emphasized that the lesion "does not in itself interfere with cardiac function but is nevertheless of serious significance on account of the fact that it is liable to become the seat of a subacute infective process." Cyanosis, clubbing of the fingers and evidence of aortic valvular stenosis are usually absent. In our case the subaortic ridge escaped but the aortic valve and the aortic intima were the seats of bacterial infection.

Summary

The example of subaortic stenosis forming the basis of this report brings the total of reported cases to thirty-one. There is evidence suggesting, but not proving, that in this instance the original process was acquired. Bacterial infection was present on the aortic valve but not on the subaortic ridge when the patient died at the age of 26 years. The clinical and pathologic aspects of this case are presented and the incidence and characteristics of subaortic stenosis are discussed.

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DESCRIPTION OF PLATE

Plate 59

Fig. 1. Photograph of left ventricle and aortic valve showing clearly the shelflike, annular stenotic ring below the aortic valve. The dark, oval, ragged areas on the right posterior aortic valve cusp and aortic intima are ulcerative vegetations.

AMERICAN JOURNAL OF PATHOLOGY. VOL. XVIII



Subaortic Stenosis

Mason and Hunter

MICROFILARIAL GRANULOMATA OF THE SPLEEN*

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Filarial lesions have been noticed very rarely in autopsies performed in Bombay. In an analysis of over 5,000 autopsies performed at the King Edward VII Memorial Hospital, Bombay, no such lesions could be discovered. However, experience derived from the out-patient department and the wards of the hospital seemed to suggest that filarial infection was not uncommon. It was well recognized that the presence of a filarial worm in the body did not necessarily mean that a pathological lesion resulted in the tissues. The female worms lying in the deep lymphatics did not produce changes sufficiently marked to be detected unless an autopsy was carried out with a view to detecting the localization of the worms in the different parts of the body. If lesions such as elephantiasis, lymphadenopathy or chylous effusions in serous sacs were present, their filarial nature was immediately recognized. During the course of the last 2 years we have encountered lesions in the spleen which could be readily detected macroscopically and which appeared to be microfilarial in origin.

The fecundated female worm lying in the deeper lymphatics gives rise, periodically, to broods of larvae which enter the general circulation to be distributed to different parts of the body. They have been observed in the capillaries of many organs such as the liver, spleen, kidneys and lungs. Manson,[1] the discoverer of filarial disease, stated: "The healthy fully formed microfilariae—that is to say the embryonic filariae which by means of the microscope we see in the blood are, so far as we can tell, particularly harmless." Being harmless it was believed that they did not produce any lesions in the body. Our observations now present evidence that microfilariae are not always innocuous and that they are capable of producing lesions in deep organs such as the spleen.

MATERIAL

Our material consisted of eleven specimens of spleen, obtained at autopsy, from patients in the King Edward VII Memorial Hospital, Bombay, in which multiple nodules were found in that viscus. Table I gives the duration of hospitalization and the clinical and postmortem diagnoses of the cases. It shows that death was due to injury in

* Received for publication, March 10, 1941.

more than half of these patients and that their stay in the hospital before death varied from a few hours to 3 or 4 days. The significance of these findings is discussed later.

Observations

Macroscopical Appearances of the Lesions in the Spleen

The spleens bearing the lesions were largely normal in size and weight. Table II gives the age of each patient and the weight of the spleen. The spleen of case no. 4 was abnormally large because of an accompanying lobar pneumonia. The resulting gray splenic tumor gave rise to the increase in weight and not the nodules which are to be described.

Table I

No.	Duration of hospitalization	Clinical diagnosis	Postmortem diagnosis
1.	2 days	Paraplegia	Transverse myelitis
2.	½ hour	Head injury	Fracture, skull
3.	1½ hours	Multiple fractures	Multiple fractures; shock
4.	Few minutes	Lobar pneumonia	Lobar pneumonia
5.	4 days	Head injury	Multiple fractures
6.	1 day	Catarrhal jaundice	Subacute atrophy, liver
7.	10 hours	Uraemia	Chronic nephritis; uraemia
8.	Few minutes	General peritonitis	Duodenal perforation
9.	Few hours	Multiple fractures	Multiple fractures
10.	Few hours	Head injury	Fracture, skull
11.	Few hours	Fracture and dislocation of spine	Multiple fractures

Table II

Serial No.	Age of the patient	Sex	Weight of the spleen	Size of the organ
	(years)		(gm.)	
1.	65	M	150	Normal
2.	40	M	90	Normal
3.	40	M	120	Normal
4.	35	M	540	Enlarged
5.	50	M	60	Small
6.	25	F	180	Normal
7.	50	M	...	Little larger
8.	15	M	...	Normal
9.	6	F	90	Enlarged
10.	8	M	45	Normal
11.	50	F	50	Smaller

In these spleens nodules could be felt at the surface, as seen in Figure 1. They were smooth and rose a little above the surface. They were a little firmer than the rest of the splenic tissue and their size varied from 2 to 25 mm. Usually they were multiple, but occasionally only a single nodule was present. On the cut surface the nodules presented a reddish or reddish brown appearance quite distinct from the chocolate colour of the splenic parenchyma. They were sharply circumscribed and occasionally two or three of them merged into one

five cases from which the kidneys were available for histological examination, four showed microfilariae in their substance, caught in the glomerular capillaries. In one case numerous glomeruli appeared larger than normal and all of these contained microfilariae. This patient had died of chronic nephritis and uraemia. Before death the urine contained albumin and casts. Histological examination of the kidneys gave evidence of chronic nephritis, but the glomeruli in which microfilariae were caught did not show any evidence of such inflammatory changes as exudate in the Bowman's capsular space or cellular infiltration inside or outside of the glomerular tuft. Whether microfilariae in a large number of glomeruli contributed mechanically to the final outcome of the case is difficult to determine.

Microfilariae Ante Mortem

In only one of our cases were the microfilariae detected in the blood during routine examination: a patient with deep jaundice who died in the hospital as a result of subacute atrophy of the liver and cholaemia. No filarial lesions were discovered at autopsy and subsequent microscopical examination of the liver and other organs, except the spleen, did not show microfilariae.

Discussion

We have been unable to discover any reference in the literature to lesions similar to those described in this paper. The nearest description of microfilarial lesions which we were able to trace was by Bonne.[2] He has described an infestation of splenic tissue with microfilariae in a Javanese male, who was killed in a motor accident. Microfilariae were discovered during a routine histological examination of the internal organs. Nothing was known about the presence of microfilariae in the patient's blood. The other internal organs did not show microfilariae. The spleen had reacted with an extraordinary degree of eosinophilia and some peculiar giant cells were noted surrounding the terminal ends of the larvae. The extreme eosinophilia, for which no other explanation was available, was believed to have some connection with the presence of microfilariae. The process was considered as either an undescribed phase in the life cycle of one of the common filarial species or a manifestation of an unusual filarial parasite. Altogether this condition was in many ways comparable to an eosinophilic microfilarial manifestation in lymph glands described by Meyers and Kouwenaar.[3]

The microscopical characters of the lesions described by Bonne[2] more or less resemble those described by us. From the summary of

number of microfilariae varied considerably. In some, every field under a one-sixth inch objective showed a fair number, while in others a prolonged search had to be carried out to locate a single microfilaria. Their number could not be associated with any particular histological appearance.

The microfilariae in cut section were distinctive. Most of them showed a clear space about the segment (Fig. 7). The walls of the body of the microfilaria were parallel and the interior contained a series of granules. Unless these were present it was difficult to identify the structure as a microfilaria. In our preparations we were not able to obtain an entire organism, the longest segment seen being 108 by 5 μ (Fig. 8). From histological sections alone it was difficult to identify the species of the microfilaria,* which might be either of the two species (*Wuchereria bancrofti* and *W. malayi*) known to produce infection in India. As seen in Figure 7, the granules in these microfilariae were large, separate and easy to count. Before attempting to ascertain the species it would be necessary to obtain entire specimens of microfilariae by teasing fresh nodules.

Microfilariae in Other Organs

In those cases in which microfilariae were present in nodules in the spleen, search was made for them in other organs (Table III). Unfortunately, tissues from all the organs were not available for study. Microfilariae were found to be present in the capillaries of the heart,

TABLE III

The Occurrence of Microfilariae in Various Organs

Serial no.	Spleen (no. of microfilariae seen)	Other organs from which sections were available	
		Microfilariae present	Microfilariae absent
1.	Large number	Kidney, heart and pancreas	
2.	Few		Liver
3.	Large number		
4.	Large number	Kidney	Gallbladder and liver
5.	Few		Kidney, lung, liver and heart
6.	Few (long)	Kidney and lung	Liver, heart and pancreas
7.	Moderate	Kidney (large number), liver, brain, testis and epididymis	
8.	Moderate	Liver	
9.	Moderate		Brain and ovary
10.	Moderate	Lung (large number)	
11.	Moderate		Liver and heart

lungs, kidneys, liver, brain, testis and epididymis without any reaction around them. In one case sections from kidney, lung, liver and heart were available but microfilariae were not found. However, in

* The microfilaria have since been identified as *Wuchereria bancrofti.*

SUMMARY

Multiple gross nodular lesions seen in the spleens of 11 patients are described. Microscopical examination showed them to contain microfilariae in every case. Evidence has been presented to show that these granulomata are microfilarial in origin.

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his article it appears that he did not notice gross nodular lesions such as those described by us. His finding of microfilariae in the sections was accidental, while we were led to search the nodules because of the presence of foci of eosinophilic infiltration. He was not able to find microfilariae in other organs. In some of our cases microfilariae were seen in organs other than the spleen.

We have considered the possibility that in the lesions described by us the presence of microfilariae was accidental, and that the nodules were due to some other etiological factor. This appears to us unreasonable in view of the fact that the microfilariae appeared to be concentrated only in the nodules, whereas in the surrounding splenic tissue they were rare and scattered, and were lying in the lumina of the blood vessels. In the nodules the reaction was unmistakably directed towards a limitation and possibly towards destruction of the numerous larvae that were present. In some of our sections microfilariae are seen in various stages of disintegration, a finding which indicates that their presence was not accidental.

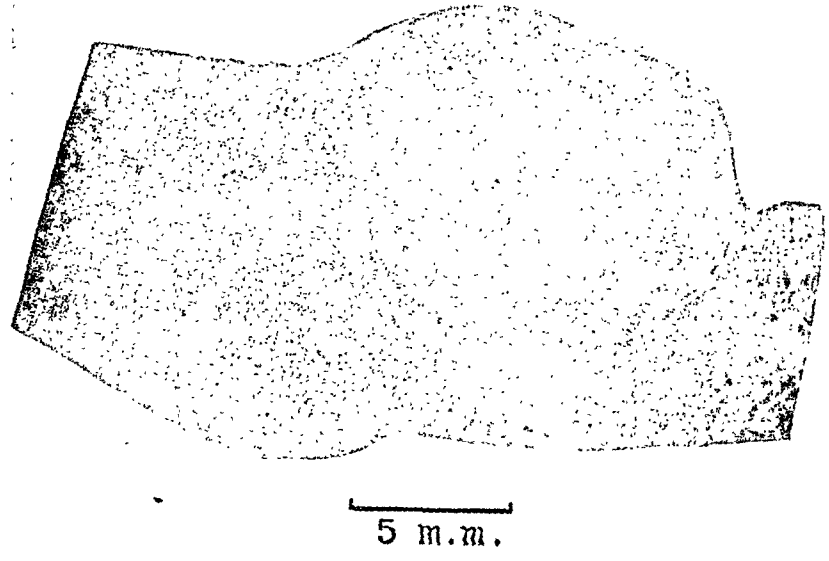
In the case described by Bonne,[2] and in many of those described by us, death was accidental. The duration of stay in the hospital was very short, varying from a few minutes to 3 or 4 days. This might be significant because it was only in such cases that lesions of a granulomatous nature which would ultimately become patches of fibrosis were likely to be encountered. This, we believe, was probably one of the reasons why the lesions described by us were not detected before. The spleen is richly endowed with reticulo-endothelial tissue which plays an important rôle in phagocytosis and the destruction of any particulate material. It is quite conceivable that the nodules are an attempt on the part of the splenic tissue to get rid of dead microfilariae. From the staining character of the microfilariae in sections, not all of them appeared to be dead, although there was no doubt that some were in various stages of disintegration. The presence of living microfilariae in the circulating blood in one of our cases militates against the hypothesis that all the microfilariae were dead and that this was one of the methods by which they were being disposed of from the body.

As previously stated, the nodules varied greatly in regard to their content of microfilaria (Table III). The number of microfilariae present probably depends on the age of the lesion. In an old and fibrosed lesion one would expect to find a small number of microfilariae, while in a young lesion they are likely to be numerous. The histological characters of the lesions supported this view.

1



3



2



4



Dhayagude and Amin

Microfilarial Granulomata of the Spleen

DESCRIPTION OF PLATES

PLATE 60

FIG. 1. Photograph of a specimen of a spleen (case no. 1) showing a nodule projecting above the surface. A small section of the spleen shows the appearance of another nodule on cut surface and a contrast in its appearance as compared with the surrounding splenic tissue.

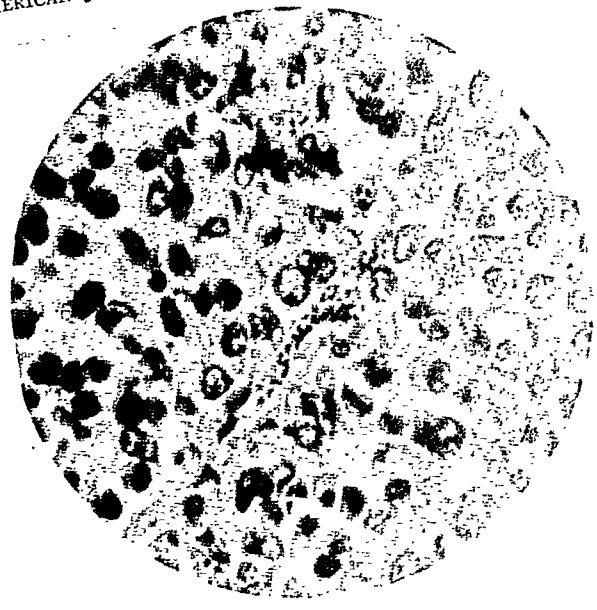
FIG. 2. Photograph of a preserved specimen showing multiple nodules projecting above the surface.

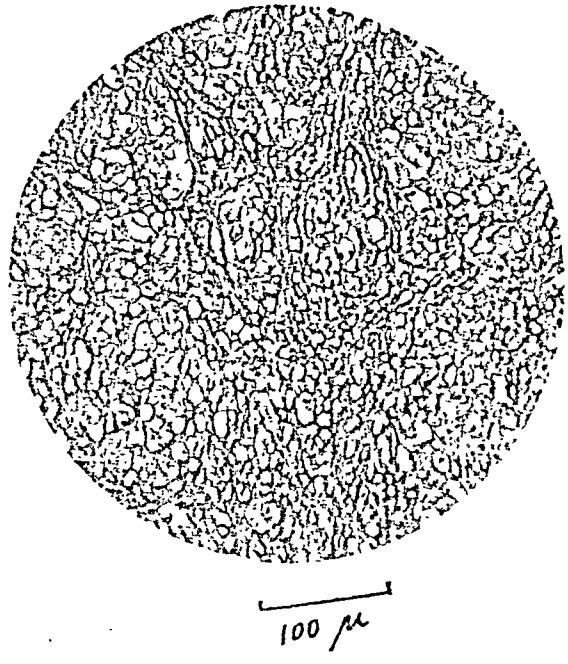
FIG. 3. A low power view of a section (stained with haematoxylin and eosin) through two large-sized nodules in the spleen.

FIG. 4. A low power view of the nodule showing a slitlike space and the close texture of the nodule.

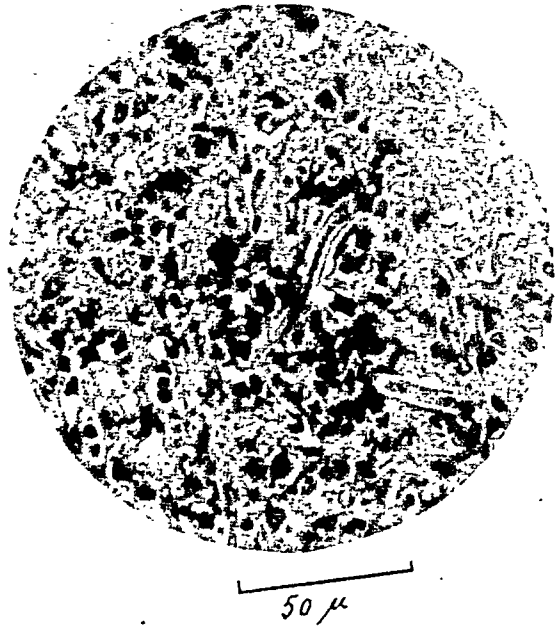
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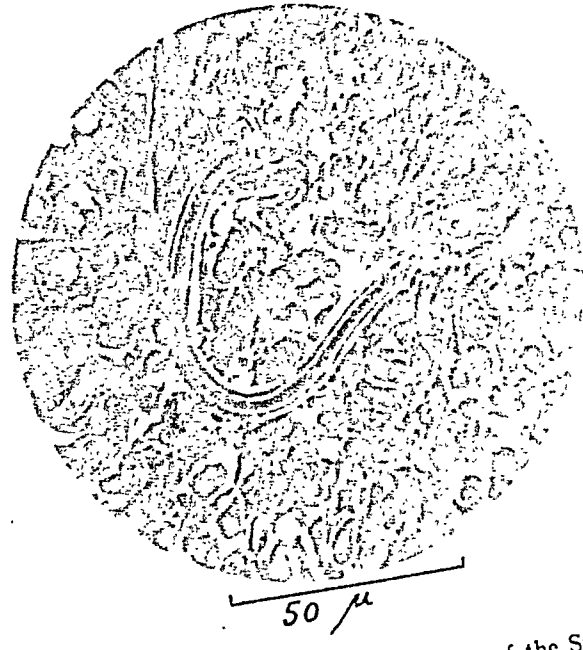
5





7





Microfilarial Granulomata of the Spleen

Dhayagude and Amin

PLATE 61

FIG. 5. A photomicrograph with oil immersion lens taken to show the eosinophile cell infiltration around the microfilaria which is seen in the centre. The cells with dark staining cytoplasm are the eosinophiles.

FIG. 6. Shows the increase in the reticular tissue as demonstrated by Foot's method of staining.

FIG. 7. Shows two microfilariae in cut sections embedded in a large amount of inflammatory cellular reaction.

Shows a large segment of a microfilaria in which the characters of the nuclei are well demonstrated.

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THE AMERICAN JOURNAL OF PATHOLOGY

VOLUME XVIII MAY, 1942 NUMBER 3

STUDIES ON LEUKOCYTOSIS *
III. HOURLY DETERMINATIONS OF THE MATURITY OF NEUTROPHILS OF NORMAL RABBITS

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In connection with studies on leukocytosis in rabbits, the question has arisen whether the maturity of polymorphonuclear neutrophilic leukocytes (amphophils) varies from hour to hour during the day. As a review of the literature shows no reported studies of this variation, results are presented of 21 such determinations made on 20 rabbits.

PROCEDURE

Total and differential leukocyte examinations were made at hourly intervals, at approximately the same hours during the same period of the day, of 20 adult normal rabbits, studied during the period from May 24, 1939 to December 30, 1940. These results and the various general procedures involved in leukocyte determinations, such as feeding, housing, selection of animals and obtaining of blood samples, have been described previously.[1] Special consideration was given to the exact duplication of these methods and to all other procedures to ensure the utmost accuracy of the results.

The method used in classifying neutrophils is based on a report by Pons and Krumbhaar,[2] the cells being arranged in four groups according to the modification of Hunt and Weiskotten,[3] as follows:

Group I. Cells with the nuclear material in one mass, round, oval, or indented not more than one-half through its width.

Group II. Cells with the nuclear material not divided into segments, but it may be lobed, spiral, looped, rosette-shaped or variously irregular.

* Aided by a grant from the Francis Hendricks Research Fund of Syracuse University College of Medicine, Syracuse, N. Y.
Received for publication, August 21, 1941.

absolute numbers of neutrophils present in the circulating blood were very low (*e.g.*, 200 to 300 per cmm.). Under such circumstances the entire surfaces of from one to three smears have been examined. As repeated comparisons of the results of classifications of 50 successive neutrophils on the same smear have shown a close correlation, the results when at least 50 neutrophils have been examined were felt to be sufficiently accurate to be acceptable. Cooke and Ponder [5] stated that the probable error of the mean was approximately ± 0.05 when 100 neutrophils were counted, and ± 0.07 when 50 cells were counted.

Neutrophils in which the nuclear masses were not stained well or were not clearly outlined, were discarded. The number of discarded neutrophils was less than 5 per cent in any experiment. Care was taken to distinguish between poor staining in general and the irregular and atypical staining which sometimes is evident in non-filamented neutrophils.

The thinness of the narrow filament between nuclear parts in neutrophils of groups III and IV was usually quite characteristic. We have used Cooke's [5] criterion on nuclear lobulation: "If there is any band of nuclear material except a chromatin filament connecting the different parts of a nucleus, the nucleus cannot, for the purpose of the count, be said to be divided." If any nuclear detail caused the question to arise in which of two groups a cell should be classified, the cell arbitrarily was placed in the group "to the right." Such a procedure was occasionally, but not frequently, necessary.

The weighted mean, as described by Cooke and Ponder,[5] was used to express the maturity of 100 neutrophils by a single figure. To determine the weighted mean, the number of cells in each group is multiplied by the number of the group, all results are added and this sum is divided by the total number of cells counted. Thus the count:

I	II	III	IV
0	31	34	35

gives a weighted mean of 3.04, that is, the average age of these 100 neutrophils is group 3.04. As Cooke and Ponder have pointed out, the weighted mean is a very sensitive index of the nuclear classification, for a difference of one point in the second decimal place means that one neutrophil has been moved to the group either below or above its original group.

Results

Neutrophilic weighted means of successive hourly counts in 21 experiments on 20 rabbits are shown in Table II, and typical hourly curves for 4 of these animals are illustrated in Chart 1. Study of Table II

Group III. Cells with the nuclear material showing segmentation into two parts which are either entirely separate (as viewed in the smear), or connected by a narrow filament.

Group IV. Cells with the nuclear material showing segmentation (as in group III) into more than two parts.

The relation of this classification to other classifications of neutrophils by the appearance of the nuclear material is shown in Table I. It is seen that groups I and II and groups III and IV comprise non-filamented and filamented neutrophils respectively. While further subdivision of the group IV cells, dependent upon the number of nuclear parts (more than two), might seem advisable, in our experience such finer classification is not practical because in many neutrophils the nu-

TABLE I

Comparison of Methods of Classifying Neutrophilic Maturity by Nuclear Appearance

Method of classification	Type of cell of neutrophilic series					
	Non-filamented cells		Filamented cells			
	Myelocyte, metamyelocyte	Nonsegmented, band, staff or stab forms; "young neutrophils"	Segmented forms*			
			2 lobes	3 lobes	4 lobes	5 lobes
Arneth count[4]	I	I	II	III	IV	V
Polynuclear count (Cooke and Ponder[5])	I	I	II	III	IV	V
Method used in present study	I	II	III	IV	IV	IV

* Our criterion of segmentation, like that of Cooke and Ponder,[5] is more strict than the criterion of Arneth.[4]

clear parts are not separated sufficiently to show more than three parts, but are adjacent to or superimposed on each other.

Hunt and Weiskotten,[3] using this method in a study of the maturity of rabbit neutrophils (amphophils) liberated from a regenerating bone marrow, concluded that a "shift to the left" in the count (increase in percentage of the simpler formed nuclei—groups I and II) actually indicated a relative increase in the number of young or more immature neutrophils in the circulating blood.

During the course of various studies on leukocytosis involving neutrophilic nuclear classifications, approximately 60,000 neutrophils have been examined and grouped.

With a few exceptions, at least 100 neutrophils have been examined and classified for each determination. These exceptions in which at least 50 and usually more neutrophils were examined, occurred when blood films were very thin or smears were taken at times when the

There were no evident variations dependent upon seasonal changes or the sex of the experimental animals.

The percentages of non-filamented neutrophils (groups I and II) ranged from 11 to 48, with a mean of 28.5, median of 29 and mode of 29. Those animals having the lowest and highest non-filamented neutrophil percentages also maintained the same general percentages throughout the successive hours of counting. Only two group I neutrophils were observed in classifying approximately 14,000 neutrophils.

In Table III are listed single neutrophilic maturity counts of rabbit B at approximately the same time of day (9:45 a.m.) on different days during a 23-month period. The data presented in this table are typical of those for the other rabbits in this series. It has been our experience that while the level of the neutrophilic weighted mean in a rabbit may

TABLE III

Neutrophilic Maturity Classifications of Rabbit B at Approximately the Same Time of Day on Different Days

Date	Neutrophilic classification				
	Group I	Group II	Group III	Group IV	Weighted mean
1-17-39	0	15	45	40	3.25
5-24-39	0	19	39	42	3.23
12- 5-39	0	15	40	45	3.30
12-29-39	0	14	34	52	3.38
3-28-40	0	31	38	31	3.00
7-17-40	0	19	40	41	3.22
9- 5-40	0	16	36	48	3.32
12-14-40	0	22	36	42	3.20

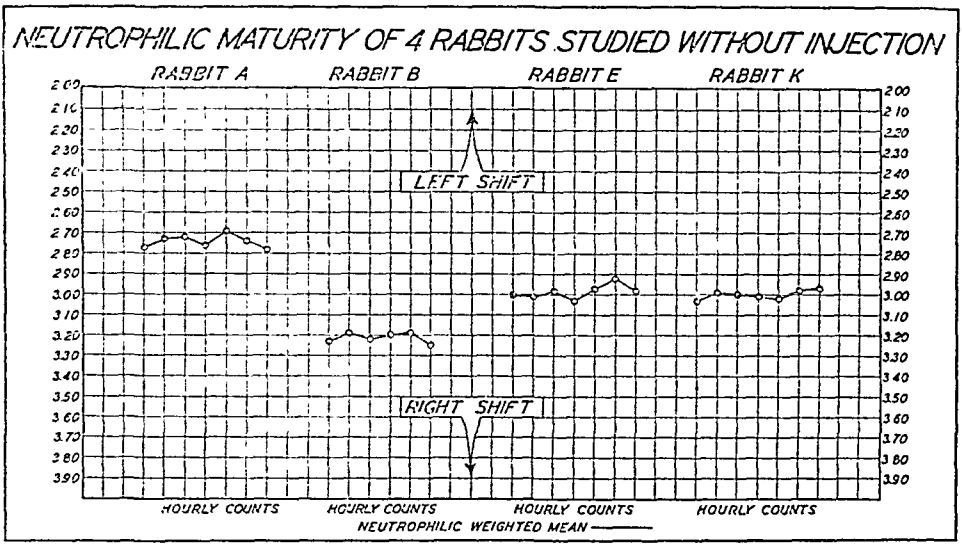
vary slightly over a long period of time, the relative constancy of the mean, established for any particular daily period of study, is maintained during this period of study.

DISCUSSION

The striking daily constancy of the weighted mean for each animal at its particular level seems to indicate that, as in a study of the hourly differential leukocyte picture during the same period, general trends should be established for each rabbit. After this, we should have every reason to expect very little shift to the "left" or "right" during the succeeding 6 hours. The maximum variation of the weighted mean in any experiment was 0.23. Cooke and Ponder,[5] classifying 1000 neutrophils of one blood film, observed a maximum variation of their weighted mean of 0.28.

Previous studies [1] have shown that there is a tendency for the absolute numbers of neutrophils in rabbits to increase slightly to moderately

CHART 1



shows that each rabbit on its particular day of counting seemed to have a rather characteristic weighted mean, some more "to the left" than others. Regardless of the value of this weighted mean, there was no apparent tendency for the mean to shift to the "left" or "right" during successive hourly counts of this period of study. Chart 1 illustrates the constancy with which the initial average neutrophilic maturity was maintained during the period of counting. Charts of the remaining 17 rabbits showed similar curves.

TABLE II

Neutrophilic Weighted Means of Successive Hourly Counts on 21 Rabbits

Rabbit letter	Weighted means of successive hourly counts							Average weighted mean
	1	2	3	4	5	6	7	
A	2.77	2.73	2.72	2.76	2.69	2.74	2.78	2.74
B	3.23	3.19	3.22	3.20	3.19	3.25		3.21
B	3.30	3.39	3.44	3.40	3.34	3.29	3.31	3.35
C	3.14	3.15	3.09	3.08	3.08	2.99		3.09
D	3.15	3.14	3.08	3.08	3.07	3.04		3.09
E	3.00	3.01	2.98	3.03	2.97	2.92	2.98	2.98
F	3.10	3.01	3.10	3.08	3.05	3.04	2.98	3.05
G	2.93	2.93	2.96	2.96	3.02	2.93	2.97	2.96
H	3.14	3.09	3.09	3.08	3.03	3.03	2.99	3.06
I	2.96	3.00	2.95	2.99	2.96	3.00	2.98	2.97
J	3.02	3.07	3.06	3.00	2.98	2.98		3.02
K	3.03	2.99	3.00	3.01	3.02	2.98	2.97	3.00
L	2.75	2.82	2.77	2.76	2.77	2.79	2.84	2.79
M	2.96	2.97	3.02	3.01	2.96	2.91	2.99	2.97
N	3.30	3.20	3.16	3.21	3.12	3.21	3.18	3.20
S	3.21	3.16	3.28	3.11	3.13	3.03	3.17	3.15
T	3.23	3.26	3.18	3.00	3.12	3.04	3.10	3.13
W	2.83	2.95	2.85	2.93	2.89	2.92	2.90	2.90
X	3.16	3.04	3.11	3.08	3.04	3.16	3.05	3.09
Y	3.02	2.98	3.06	2.97	3.03	3.03	3.02	3.02
Z	2.94	2.94	2.88	2.88	2.92	2.94	2.93	2.92

STUDIES ON LEUKOCYTOSIS *

IV. The Neutrophilic Maturity Following Intravenous Injection of Supernatant Fluid from a Sterile Exudate (Rabbit)

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(From the Department of Pathology, Syracuse University College of Medicine, Syracuse, N. Y.)

Previous studies [1] have indicated that an absolute neutrophilic leukocytosis regularly occurs in rabbits several hours following the intravenous injection of supernatant fluid from a sterile, sodium chloride-induced exudate from the peritoneal cavity of a rabbit. Apparently some substance or substances, not sodium chloride, present in these exudates, produced these neutrophilic leukocytoses.

While it is possible that a neutrophilic leukocytosis may result from an altered distribution of neutrophils in various organs, it is generally agreed that an increase in the number of more immature neutrophils in the circulation (so-called "left shift") can result only from release of younger neutrophils from the bone marrow. It would seem that studies of the neutrophilic maturity following intravenous injection of supernatant fluid from a sterile exudate might give more information on this point.

In the present study, hourly variations in neutrophilic maturity and total and differential leukocyte counts were determined for 11 rabbits, each studied at the same hourly intervals during the same daily period, without injection and before and after single and repeated injections of 5 cc. of supernatant fluid from a sterile rabbit exudate. Four of these rabbits received in addition single injections of 25 cc. of supernatant fluid. Similar studies were made on 5 of these rabbits following single or repeated control injections of 5 cc. of sterile 0.9 to 0.78 per cent sodium chloride solution.

Procedure

The procedures involved in the selection, feeding and housing of these rabbits; the determinations of total and differential leukocyte counts and variations in neutrophilic maturity, and the preparation, characteristics and injection of the sterile rabbit exudates have been described

* Aided by a grant from the Francis Hendricks Research Fund of Syracuse University College of Medicine, Syracuse, N. Y.

An abstract of some of the results of this investigation was read by title at the Forty-First Annual Meeting of the American Association of Pathologists and Bacteriologists, New York City, April 11, 1941, and was read at the organization meeting of the American Federation for Clinical Research, May 5, 1941, and at the annual meeting of the New York State Association of Public Health Laboratories, May 19, 1941.

Received for publication, August 21, 1941.

during the afternoon hours. If we consider any neutrophil count, quantitative or qualitative, to represent the balance between constant production and constant destruction of neutrophils, the lack of "left shift" accompanying the frequent afternoon neutrophilic increases would seem to indicate that the ratio of appearance of younger neutrophils in the blood to the disappearance of older neutrophils from the blood was balanced or constant.

The scarcity of group I cells (0.014 per cent) seems to indicate that under normal conditions in these rabbits, with very rare exceptions, cells of the neutrophilic series were not discharged from the bone marrow into the circulating blood unless they were of a certain maturity, *i.e.*, more mature than the metamyelocytic stage (group I).

Summary

Variations of maturity of polymorphonuclear neutrophilic leukocytes (amphophils), expressed as a single figure, the weighted mean, are presented for 20 apparently normal rabbits, studied at successive hourly intervals during a 6-hour daily period.

Each rabbit seemed to have a characteristic degree of neutrophilic maturity on the day on which counting occurred. Regardless of this degree, neutrophilic maturity was relatively constant during the successive hours of counting.

In these rabbits, with very rare exception, only cells of the neutrophilic series which were more mature than metamyelocytes (group I) were observed in the circulating blood.

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"left shifts" and the duration of the exudate or the particular animals in which the exudates were produced. When a supernatant fluid fraction of the same exudate was injected into two rabbits, there was no evident correlation between the neutrophilic changes in the two rabbits.

As a decrease of the weighted mean may result from a relative de-

TABLE I

Hourly Neutrophilic Weighted Means in 25 Experiments on 11 Rabbits Receiving Intravenous Injections of 5 cc. of Supernatant Fluid

Rabbit letter	Type of injection	Hourly neutrophilic weighted means							Time after injection at which count no. 2 was taken (hours)*
		1	2	3	4	5	6	7	
B	Single	3.38	3.10	2.89	2.94	2.89	2.86	3.13	1
B	Single	3.00	2.78	2.72	2.59	2.49	2.56	2.85	1
B	Repeated	3.32	3.00	2.56	2.31	2.32	2.29	2.18	½
F	Single	3.08	3.07	2.58	2.56	2.47	2.53	2.83	½
F	Single	2.99	2.71	2.59	2.55	2.69	2.77	2.88	1
F	Repeated	†	3.12	2.84	2.68	2.89	2.91	2.68	½
I	Single	2.85	2.60	2.62	2.55	2.57	2.78	2.80	1
I	Single	2.76	2.58	2.49	2.61	2.59	2.66	2.88	5/6
I	Repeated	3.16	3.18	2.88	3.04	3.03	3.05	2.94	½
M	Single	2.84	2.55	2.51	2.46	2.48	2.45	2.85	1
M	Repeated	3.02	2.90	2.65	2.45	2.30	2.26	2.15	½
N	Single	3.14	2.82	2.72	2.65	2.71	2.84	2.92	1
N	Repeated	3.18	2.87	2.68	2.50	2.36	2.30	2.23	½
S	Single	3.02	3.02	2.73	2.64	2.71	2.86	2.94	½
S	Repeated	3.06		2.30	2.43	2.27	2.10	2.19	‡
T	Single	3.12	2.90	2.81	2.71	2.77	2.79	2.89	½
T	Repeated	2.96		2.50	2.42	2.42	2.30	2.33	‡
W	Single	3.07	2.84	2.72	2.64	2.82	2.86	2.94	½
W	Repeated	2.90	2.70	2.38	2.22	2.28	2.20	2.26	½
X	Single	2.92	2.85	2.82	2.69	2.83	2.93	2.89	½
X	Repeated	3.05	2.76	2.38	2.28	2.28	2.34	2.26	½
Y	Single	2.97	2.80	2.78	2.67	2.73	2.74	2.84	½
Y	Repeated	3.04	2.82	2.44	2.37	2.27	2.36	2.23	½
Z	Single	2.83	2.80	2.63	2.52	2.61	2.60	2.70	½
Z	Repeated	3.00	2.80	2.43	2.14	2.23	2.27	2.26	½

* Hours after first injection, in the case of repeated injections.
† No determination (unsatisfactory stain).
‡ No. 3 count 1¾ hours; no. 4 count 2½ hours.

crease in the absolute numbers of older neutrophils present in the circulation, the absolute number of neutrophils in groups I, II, III and IV were calculated in all experiments. Table II shows the results of neutrophil studies of rabbit I without injection and before and after an intravenous injection of 5 cc. of supernatant fluid. This table shows that the decreased neutrophilic maturity was due to an increased number of more immature neutrophils in the circulation. The absolute numbers of more mature neutrophils seemed to remain relatively constant during the period of counting. Similar results were obtained in the other ten animals.

in detail elsewhere.[1-3] These procedures may be summarized briefly as follows: exudates were produced by the intraperitoneal injection into rabbits of 300 cc. of sterile 0.9 per cent sodium chloride solution. The fluid exudate was withdrawn 5¾ to 15½ hours later, centrifugated, and either 5 or 25 cc. of the supernatant fluid fraction immediately injected intravenously into the rabbit to be studied. In the case of repeated injections, the supernatant fluid was kept at room temperature during the period of injections.

The sodium chloride concentrations of some of these supernatant fluid fractions had previously been determined.[1] Sterile sodium chloride solutions of equivalent concentrations were injected intravenously in equivalent amounts into five of these animals.

The repeated injections consisted of four 5 cc. injections at hourly intervals beginning at 10:15 a.m. The experiments involving repeated supernatant fluid injections preceded the experiments involving single supernatant fluid injections in four animals. In the other seven animals, repeated injection experiments followed single injection experiments. All repeated control injections of saline solutions were given after supernatant fluid injections had previously been made into the same rabbits. The interval between any two experiments ranged from 14 to 140 days.

Total and differential leukocyte counts and determinations of neutrophilic maturity of these 11 rabbits were made under the varying experimental conditions at hourly intervals at the same times of day during the same 6-hour daily period. Subsequent leukocyte studies were also made at intervals varying up to 101½ hours after an injection. The weighted mean [3] was used to express the degree of neutrophilic maturity of each determination as a single figure. Every attempt was made to have all factors of these experiments constant except the amounts and types of material injected.

Results

Results are presented in Tables I to IV and Charts 1 to 4.

In a series of 25 experiments, 11 rabbits received both single and repeated injections of 5 cc. of supernatant fluid fractions of sterile rabbit exudates. A neutrophilic leukocytosis, comparable in extent and time of occurrence to those previously described,[1] occurred in every experiment. In each experiment this leukocytosis was accompanied by a decrease of the neutrophilic weighted mean, indicating a "shift to the left" or appearance of a greater proportion of younger or more immature cells in the circulating neutrophil population. The hourly neutrophilic weighted means in these 25 experiments are shown in Table I.

There was no evident correlation between these leukocytoses with

rabbit N. Chart 3 (rabbit F) shows that after repeated injections of supernatant fluid, a maximum neutrophilic leukocytosis occurred, while the degree of "left shift" was approximately the same as that observed following a single injection of 5 cc. of supernatant fluid.

CHART 1 RABBIT B

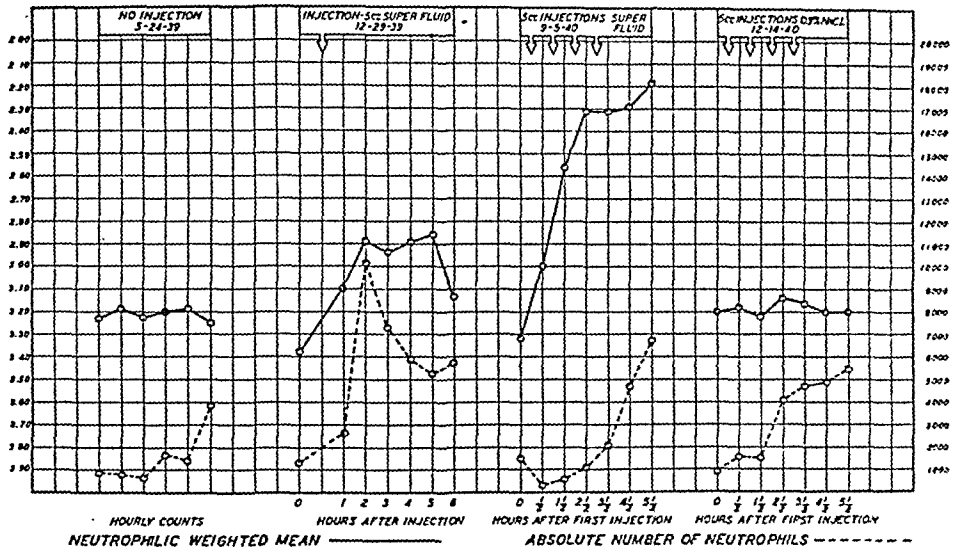


CHART 2 RABBIT N

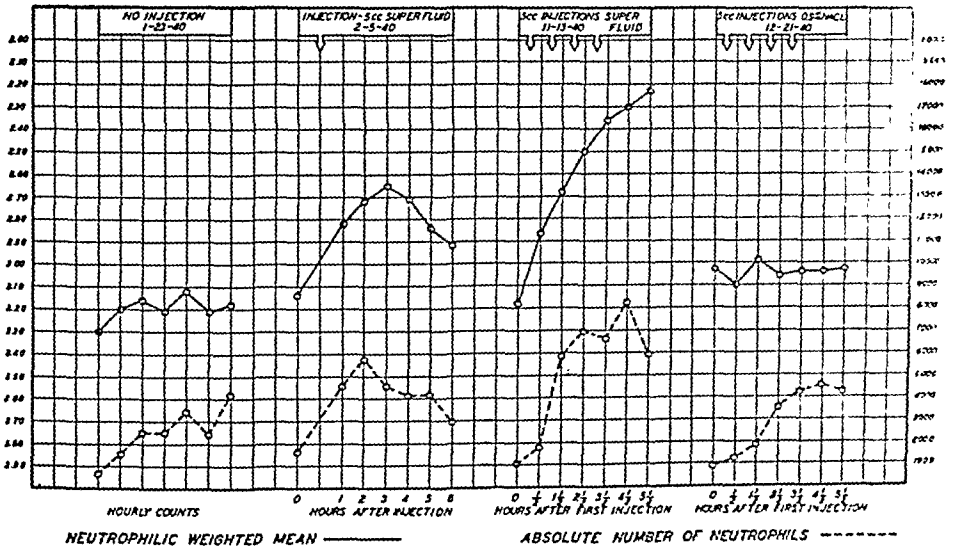


Table III shows the maximum decrease of the neutrophilic weighted mean ("left shift") which occurred in each rabbit following single and repeated injections of 5 cc. of supernatant fluid. Following single injections, the maximum "left shifts" occurred approximately 2 to 4 hours after injection. In all animals except rabbits F and I, the "left shifts" were considerably greater and tended to progress, rather than to

As a control procedure, leukocyte studies were made on five of these rabbits after single or repeated injections of 5 cc. of sterile sodium chloride solution. In every experiment the absolute numbers of neutrophils at successive hours varied no more than when studied without injection during the same daily period, and the neutrophilic maturity

TABLE II

Absolute Numbers of Neutrophils per Cubic Millimeter in Each Group of Maturity (Rabbit I)

	Time	Absolute numbers of neutrophils per cmm.	Classification of neutrophilic maturity			
			Group I	Group II	Group III	Group IV
Experiment 39.43— Dec. 11, 1939— without injection	9:45 a.m.	1,342	0	416	564	362
	10:45 a.m.	1,166	0	373	420	373
	11:45 a.m.	1,414	0	481	523	410
	12:45 p.m.	1,392	0	418	570	404
	1:45 p.m.	1,620	0	518	648	454
	2:45 p.m.	1,890	0	548	794	548
	3:45 p.m.	1,575	0	457	693	425
Experiment 40.1— Jan. 8, 1940—5 cc. supernatant fluid injected intravenously at 9:45 a.m.	9:30 a.m.	1,008	0	413	333	262
	10:45 a.m.	2,160	0	1,166	692	302
	11:45 a.m.	5,264	0	2,527	2,211	526
	12:45 p.m.	4,046	0	2,266	1,335	445
	1:45 p.m.	3,780	0	1,928	1,550	302
	2:45 p.m.	3,392	0	1,357	1,424	611
	3:45 p.m.	2,508	0	1,003	1,003	502
	12½ hours*	2,145	0	858	772	515
	24¾ hours*	1,134	0	363	567	204

* After injection.

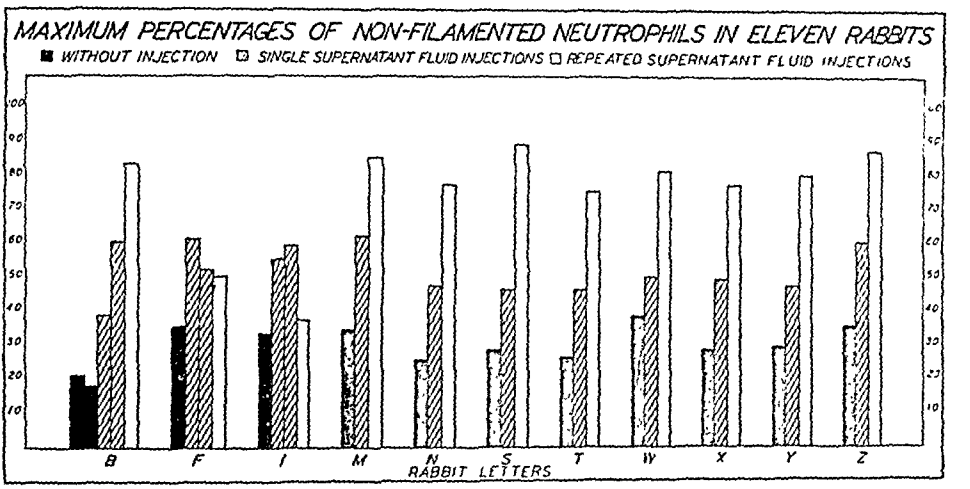
remained relatively constant. The maximum decrease in the weighted mean in any control experiment was 0.09, which was well within the limits of normal variation.

Comparison of the numbers and maturity of the neutrophils of three animals under the varying experimental conditions is possible from Charts 1, 2 and 3 (rabbits B, N and F respectively). These charts are typical of all experiments on all 11 rabbits. The dates of each experiment indicate the order in which the various procedures were performed. Chart 1 shows that the hourly neutrophilic maturity of rabbit B was relatively constant without injection and following repeated injections of 5 cc. of sterile sodium chloride solution. Neutrophilic leukocytoses and "left shifts" occurred after both experiments involving supernatant fluid injection. The "left shift" following a single 5 cc. injection reached its maximum approximately 2 hours after injection. After repeated 5 cc. injections the neutrophilic "left shift" was greater and more progressive and the neutrophilic leukocytosis somewhat more progressive. Chart 2 presents similar results of identical experiments on

level from 4½ to 24¼ hours (average 9 hours) after a single 5 cc. injection, and from 7½ to 72 hours (average 45½ hours) after the first of four repeated 5 cc. injections.

The maximum percentages of non-filamented neutrophils (groups I and II) observed in studies of these 11 rabbits without injection, and before and after single and repeated injections of 5 cc. of supernatant fluid are shown in Chart 4. It is evident that in 9 of these 11 rabbits,

CHART 4



approximately 70 to 90 per cent of the circulating neutrophils were non-filamented at the maximum of the "left shift" following repeated injections. The maximum increases in percentages of non-filamented neutrophils observed in these 11 rabbits, studied under these conditions, are presented in Table IV. Despite the large increases in percentages of non-filamented neutrophils after supernatant fluid injection, only 12 group I neutrophils (metamyelocytes) were observed during this study of approximately 41,000 neutrophils. Thus the group II cells almost exclusively constituted the non-filamented neutrophilic population in these experiments.

The neutrophilic changes following a single intravenous injection of 25 cc. of supernatant fluid were studied in four additional experiments on four of these rabbits. The neutrophilic leukocytosis which occurred in each was similar in extent and time of occurrence to the leukocytoses after single or repeated 5 cc. injections. The neutrophilic "left shifts" accompanying these leukocytoses in general were intermediate in extent between the "left shifts" following single 5 cc. and repeated 5 cc. injections. The maximum decreases of the weighted means in these four experiments ranged from 0.48 to 0.74, averaging 0.63.

peak, during the consecutive hours of observation after repeated injections. However, animals F (Chart 3) and I, although having "left shifts" which were approximately the same after both single and repeated injections, showed their highest neutrophilic leukocytoses after repeated injections. The neutrophilic maturity returned to a normal

CHART 3 RABBIT F

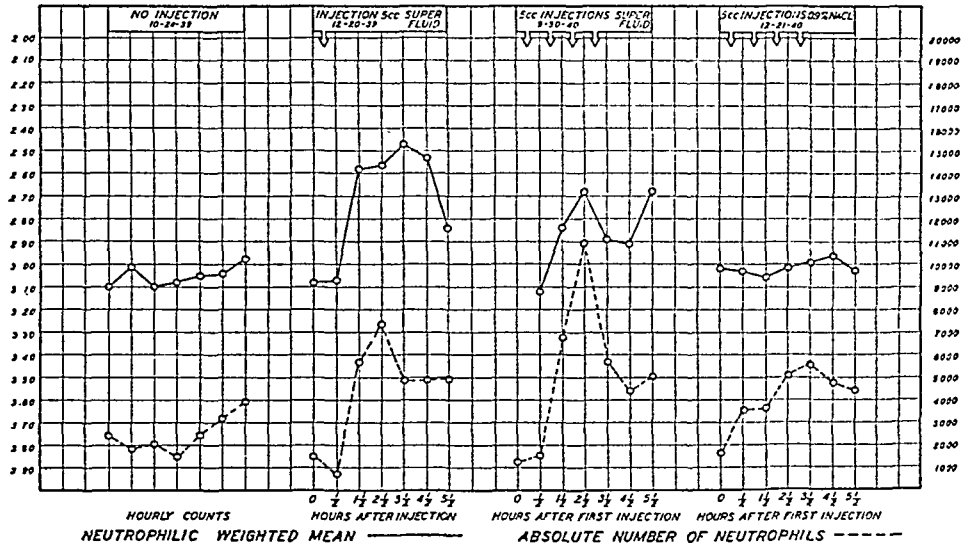


TABLE III

Maximum Decreases of Neutrophilic Weighted Means ("Left Shifts") in 11 Rabbits Receiving Single and Repeated Injections of 5 cc. of Supernatant Fluid

Rabbit letter	Single injection	Repeated injections
B	0.42 0.51	1.14
F	0.61 0.44	0.44
I	0.30 0.27	0.28
M	0.39	0.87
N	0.49	0.95
S	0.38	0.96
T	0.41	0.67
W	0.43	0.70
X	0.23	0.80
Y	0.30	0.81
Z	0.31	0.86
Average	0.39	0.76

to be present in the circulating blood. This scarcity of group I cells also has been observed in studies of these rabbits without injection.[3] Cells of the neutrophilic series more immature than metamyelocytes never were observed in the circulating blood in these experiments.

In general, the results of these experiments indicate that rabbits receiving both single and repeated injections of 5 cc. of supernatant fluid fractions, regardless of the order of the injections, had neutrophilic "left shifts" which were greater in extent and more progressive following the repeated injections. Stated in another way, following repeated injections there was a greater number of more immature neutrophils in the circulation for a longer period of time. These observations suggest that a "summation" of "shifts to the left" of the neutrophilic maturity occurred after repeated injections.

As far as these experiments are concerned, there seems to be no justification for assuming that these neutrophilic changes occurred because of conditioning of the rabbits by previous injection of supernatant fluid.

Neutrophilic "left shifts" have been reported to have followed various procedures, including the administration of bacteria,[4, 5] various split proteins,[6, 7] thyroid extract,[8] and x-ray[9] and ultraviolet irradiation.[10] Ponder and Macleod [11] presented the results of one experiment in which an approximately comparable neutrophilic "left shift" followed the intraperitoneal injection into a rabbit of 10 cc. of supernatant fluid from a peritoneal exudate induced in a rabbit by a saline solution. Menkin [12] reported that neutrophilic immaturity followed the intracardiac injection into dogs of the pleural exudate induced in a dog by turpentine. The relation of these "left shifts" reported in the literature to those observed in the present experiments is uncertain.

As has been indicated previously,[1] the chemical and physical nature of the substance or substances in these supernatant fluid fractions of sterile exudates which produced these neutrophilic changes is unknown. Routine bacteriological checking has yielded negative results. Leukocyte studies following single and repeated intravenous injections of 5 cc. portions of sterile sodium chloride solutions into these same animals would seem to eliminate the possibility that sodium chloride *per se* produced these neutrophilic changes.

Summary

Supernatant fluid fractions of centrifugated 5¾- to 15½-hour sterile peritoneal exudates from rabbits were obtained as described previously.[1] Twenty-five experiments were performed, in which 11 rabbits each received single and repeated intravenous injections of 5 cc. of these supernatant fluid fractions. In each experiment there occurred a

Discussion

Hourly determinations of the neutrophilic maturity of rabbits studied without injection have shown a striking tendency for the index of maturity to remain constant.[3] This constancy may be contrasted with the neutrophilic "left shifts" which occurred in these same rabbits following intravenous injections of supernatant fluid fractions of sterile exudates.

Table IV

Maximum Increases in Percentages of Non-filamented Neutrophils in 11 Rabbits Receiving Single and Repeated Injections of 5 cc. of Supernatant Fluid

Rabbit letter	Without injection	Single 5 cc. injection	Repeated 5 cc. injections
B	2 3	25 30	68
F	4	33 24	27
I	3	15 20	14
M	1	29	60
N	6	25	60
S	0	27	66
T	11	27	48
W	0	21	46
X	3	16	55
Y	4	24	53
Z	4	24	57
Average	3.5	24	50

Examination of Table II shows that these "left shifts" resulted from increases in the numbers of more immature neutrophils in the circulating blood. It seems more probable that this neutrophilic immaturity was effected by a release of younger neutrophils from the bone marrow, rather than an alteration of neutrophilic distribution in organs other than the bone marrow.

It would seem that a marked release of these more immature neutrophils must have occurred when marked neutrophilic "left shifts" occurred, such as after repeated injections of supernatant fluid. Despite the repeated observation that from 70 to 90 per cent of the neutrophils were more immature (non-filamented) at the maximum of the "left shift," group I neutrophils (metamyelocytes) very rarely were observed

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neutrophilic leukocytosis and a neutrophilic "left shift." These "left shifts" were due to increased numbers of more immature neutrophils in the circulating blood.

The "left shifts" following single injections tended to reach a peak 2 to 4 hours after injection. Repeated injections were followed by considerably greater and more progressive "left shifts" in 9 of the 11 rabbits. In the other 2, repeated injections were followed by considerably greater neutrophilic leukocytoses. Neutrophilic leukocytoses were approximately equal in all other experiments. The "left shifts" after single injections of 25 cc. of supernatant fluid in four additional experiments on 4 of these rabbits, in general, were intermediate in extent between the "left shifts" following single 5 cc. and repeated 5 cc. injections.

Group I neutrophils (metamyelocytes) very rarely were observed in the circulating blood, even when marked "left shifts" (70 to 90 per cent non-filamented neutrophils) were present.

Control experiments, in which 5 of these 11 rabbits received single or repeated injections of 5 cc. of sterile sodium chloride solutions of equivalent concentrations, showed no neutrophilic leukocytoses or "left shifts."

Conclusions

Some substance or substances, not sodium chloride, present in supernatant fluid fractions of sterile peritoneal exudates from rabbits, produced neutrophilic "left shifts" when injected intravenously into other rabbits. Following repeated injections of supernatant fluid, there was an apparent "summation" of these "left shifts."

These neutrophilic "left shifts" were effected by release of more immature neutrophils in greater numbers, probably from the bone marrow. In these experiments, metamyelocytes (group I neutrophils) were observed very rarely, and no cells more immature than metamyelocytes were found in the circulating blood.

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MALIGNANT LYMPHOMA *

A Clinico-Pathologic Survey of 618 Cases

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Many generic terms have been utilized to designate those maladies of the lymphatic system which are characterized clinically by progressive tumor-like enlargement of lymphoid tissue with eventual fatality, and histologically by multiplication of one or more of the elements normally present in lymph nodes to the point of destruction of the nodal architecture. Of these, "malignant lymphoma" seems to have won most general usage in this country, at least, and has the advantage of being noncommittal as to pathogenesis.

Numerous attempts to subdivide this group of diseases have been complicated by an extraordinary confusion of terminology and controversial theses. In recognition of the complexity of the problem, The American Association of Pathologists and Bacteriologists established a "Registry of Lymphatic Tumors." Although no official classification has been promulgated, the publication in 1934 [1] by the registrar at that time, George R. Callender, of a review of the Registry material tacitly set the seal of the Registry's approval upon the schematization and terminology employed. This represented in fact an attempted fusion of three classifications: (1) cytologic, based upon morphologic recognition of component proliferating cells; (2) gross anatomical, depending upon the distribution of the process throughout the organs of the body; and (3) clinical, contingent upon physical signs and hematologic manifestations. In the intervening years no revision or amplification has appeared.

In the face of such authority it is with no little hesitation that we have the temerity to offer a somewhat different classification. An honest attempt over a period of years to apply the Registry terminology to our material has convinced the staff of this laboratory that it is in many respects impractical for routine use. Any considerable experience with malignant lymphoma makes it apparent that there is no necessary correlation between cytology and distribution. Any type of cell may be associated with a localized process, with a generalized disease involving multiple organs without blood manifestations, and, in all probability, with the presence of significant numbers of the type cell in the circulating blood—leukemia, in fact. It must be conceded, however, that

* Received for publication, August 16, 1941.

tiated lesions is simple; with less differentiation it becomes somewhat more difficult but is still feasible, and in a small number (6 per cent) of poorly differentiated lesions it becomes exceedingly difficult.

To those who may share our beliefs and regard all members of this group of diseases as neoplastic entities, the proposed classification has the advantage of conformity with the accepted usages of oncology. Classification of neoplastic disease is fundamentally cytologic, resting primarily on identification of the type cell with secondary qualification dependent on degree of differentiation rather than upon the distribution of metastasis. If such classification has more than academic value in the field of malignant lymphoma, it should show a reasonable degree of correlation with the clinical course of the disease. Such correlation is, we believe, demonstrated in Part II of this study.

The material included in this survey consists of all examples of malignant lymphoma passing through the Pathology Laboratory of the Massachusetts General Hospital in the 20-year period from January, 1917, to December, 1936, from which satisfactory histologic sections were available for personal study. This comprised 135 autopsies and 580 biopsies obtained from a total number of 618 patients. The results of a histologic survey constitute the first part of this paper. Clinical data from 545 of these have been analyzed and form the basis for Part II (Text-Fig. 1).

Part I. Histologic Studies and Classification

It proved easily possible to divide the great majority of the specimens into seven categories. The nomenclature adopted and the number of cases of each type were as follows:

1. Stem cell lymphoma, 56 cases } "Reticulum cell sarcoma"
2. Clasmatocytic lymphoma, 71 cases }
3. Lymphoblastic lymphoma, 85 cases
4. Lymphocytic lymphoma, 135 cases
5. Hodgkin's lymphoma, 193 cases
6. Hodgkin's sarcoma, 36 cases
7. Follicular lymphoma, 42 cases

A distinct division can be made between the first four types and the last three. The histologic pattern of the former is comparatively simple whereas that of the latter is relatively complex. Except in the cytologic peculiarities of the type cell the general structure of members of the first four categories is so similar that many features can best be described commonly for the entire group. Common to all is the tendency of proliferating cells to encroach upon, obscure and finally replace the

many authorities will fail to recognize such relations in some of the subtypes, notably Hodgkin's disease, though Warthin [2] has recorded well documented instances. Nothing in the histologic character of an individual lesion will permit a reliable forecast as to the distribution of the process in other parts of the body. Moreover, determination of the extent and character of organ involvement is beyond the scope of ordinary clinical examination in many instances and must frequently await postmortem study, thus establishing a significant limitation, to say the least, to its clinical utility. Furthermore, another fallacy is introduced by dependence upon the results of necropsy. This shows only a single stage of the process, ordinarily, it is true, the terminal one, but not necessarily, since mechanical and surgical accidents or infection may have contributed to a lethal outcome at a comparatively early period in the evolution of the disease. More specifically, in regard to invasion of the blood stream, no constant histologic substrate permits one to predict whether or not the blood picture will prove "leukemic." Though diffuse bone-marrow involvement will be found in the great majority of leukemic patients, the marrow may be normal.[3,4] Conversely, diffuse marrow change is not infrequently found with normal blood pictures.[5,6] Still less reliable are the "leukemic" types of organ infiltration without tissue destruction such as are commonly seen in the liver, spleen and kidneys. They are frequently absent in leukemia and may be seen in typical form without leukemia. Moreover, continued observation of patients over a period of years shows significant variation in the blood picture, leukemic pictures alternating with non-leukemic ones, often quite irrespective of roentgen therapy. This feature will be dealt with in more detail in the body of this publication. Classification based upon distribution has therefore only a limited value; temporally limited because it is valid only for a given stage of the disease and must be altered from time to time in the same individual; practically limited in many instances by the impossibility of accurately determining distribution during life.

The alternative form of classification, cytologic, depends for its validity and applicability upon: (1) the constancy over months and years of observation of the reacting cell types, and (2) the possibility of histologic distinction in routine biopsy material. The first point, which is fundamental, will be made a subject for further analysis later in this paper. It is sufficient to state at this point that great constancy does in fact prevail and though variations occur they are practically limited to degree of differentiation without significant shifts between one major group and another. In respect to the second point, the applicability to routine biopsies, distinction between the well differen-

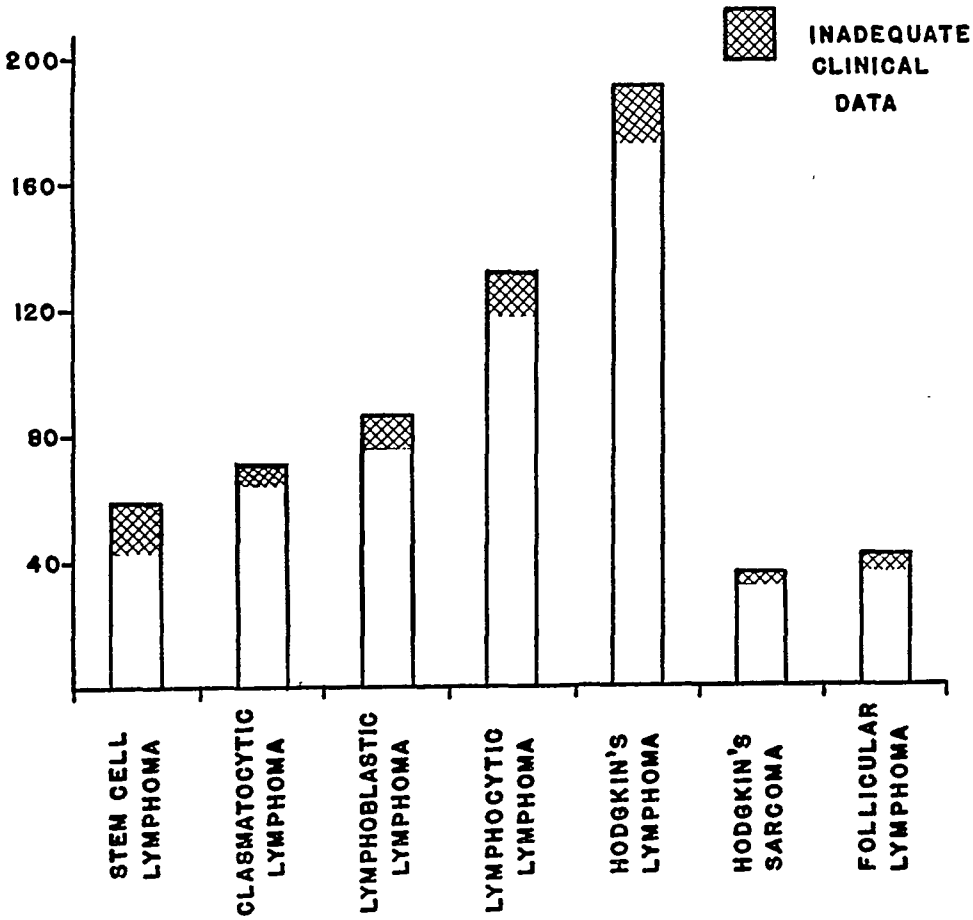
tissue. Three possibilities for their origin must be considered: (1) they are persistent elements of the original nodal tissue; (2) they represent evidences of successful, complete differentiation of tumor cells, and (3) they constitute components of an exudative reaction to the products of disintegrating tumor cells. It is probable that each of these possibilities is, on occasion, an actuality and all efforts at interpretation must be guarded.

A problem offering equal difficulty is the correct interpretation of multinucleated cells in these tumors. On the one hand is the knowledge that multinucleated "tumor giant cells" may occur in almost all types of malignant, and in a few benign, neoplasms. On the other hand is their peculiar frequency in Hodgkin's disease. We have felt that occasional multinucleated cells do not necessarily predicate a classification as Hodgkin's disease.

Cytologically, two broad lines of division in malignant lymphoma have long been recognized: Hodgkin's disease with its complicated structure, described independently by Sternberg [7] and by Reed;[8] and the simpler proliferative conditions of the lymphocytic series of cells variously termed, according to their distribution, lymphatic leukemia, aleukemic leukemia and lymphosarcoma. More recently, Roulet [9,10] has added a third type, named by him "Retothelsarkom" and generally entitled in this country, reticulum cell sarcoma. The justification of such further subdivision was immediately attested by the prompt and wide acceptance of the term throughout the world. Whether or not this last group represents a single entity may, however, legitimately be doubted in view of the wide discrepancies in the descriptions and theories of its origin which various authors have offered. A complete bibliography would be profitless, but let us examine a few examples. Ewing [11] implied that reticulum cell or large round cell sarcoma is a tumor composed of cells less mature than the lymphocyte derived from the germinal centers or pulp cords. Klemperer [12] stated that the reticulum cell is totipotential and similar to cells observed in the embryonic mesenchyme. Medlar [13] used the expressions reticulum cell and stem cell interchangeably. Rhoads [14] derived the tumor from elements of the phagocytic cell series, a view followed by Parker and Jackson.[15] The latter authors clearly distinguished reticulum cell sarcoma from the malignant type of Hodgkin's disease, but Callender [1] stated that reticulum cell sarcoma is Hodgkin's sarcoma. Roulet, himself, included under the heading of Retothelsarkom a group of lesions with a morphologic range which, judging from his descriptions and his illustrations, extends almost from one extreme to the other of the malignant lymphomas.

It is apparent that various authorities regard the type cell of this

normal nodal architecture, reconstructing the stromal framework more or less completely in the process. Tendencies to invade marginal and medullary sinuses, to migrate through the capsules and invade perinodal tissues are representative of all four and are found irrespective of the degree of localization or generalization of the disease. In nodes showing an early stage of involvement it is frequently possible to ob-



TEXT-FIGURE 1. Frequency of the subgroups by number of cases.

serve invading strands of tumor cells projecting from an established focus into the residual normal tissue of the node. Under such conditions the reticulum fibrils of the original tissue appear to be pushed aside rather than disrupted by the invading cells, resulting in a stromal condensation which occasionally simulates encapsulation. This, however, is but a transient phase and eventually, with complete invasion, all evidence of this condensed reticulum disappears and a delicate fibrillar network completely replaces the preëxisting stroma (Fig. 20).

A problem in histologic interpretation common to all members of the lymphoma group is the significance of mature lymphocytes and clasmatocytes frequently scattered in considerable numbers throughout the

usually a single prominent vesicular nucleolus. We have been able to observe no unequivocal evidence of reticulum formation by these cells under either normal or neoplastic conditions.

Cells of this type, or at any rate cells morphologically indistinguishable from them, are found in small numbers in all tumors of the lymphoma group, just as they are present in normal lymphoid tissue. In the majority of lymphomas, however, they are so rare as to escape notice unless specifically searched for. In a limited group they constitute the predominant element and it is for this group that we have proposed the name "stem cell lymphoma."

Tumors of this type occur in two forms clearly described by Ehrlich and Gerber [24] under the heading of reticular and intermediary types of lymphosarcoma. In one the neoplasm appears to consist of homogeneous-syncytial sheets (Fig. 1) and in the other of discrete cells (Fig. 2). In general, however, the fundamental similarity is so great and the two forms of growth appear simultaneously with sufficient frequency to warrant combining them as a single histologic unit. In the discrete cell type (Fig. 4) the individual cells conform closely to the description given for the stem cell of the normal lymph node, tending, however, to be larger and in a given lesion to be very uniform in size. One variation is the usual presence of a single, large, densely basophilic nucleolus. The cytoplasm is homogeneous, poorly outlined and occasionally, but rarely, exhibits particulate phagocytosis. The syncytial type which occurs much less frequently exhibits exactly similar cytologic characteristics but lacks almost entirely the separation of one cell from another (Figs. 1 and 3).

Moderate numbers of lymphocytes and monocytes are noted in some of the tumors, very few in others, particularly those of the syncytial type. Fibrosis is observed in a few of the cases but is an unusual occurrence. Reticulum is comparatively sparse and its distribution is irregular in all the tumors of this group, particularly in those of the syncytial type.

2. *Clasmatocytic Lymphoma*

In contrast to the preceding group, with which, in our estimation, it has been widely confused, the cells in these tumors simulate more or less closely normal clasmatocytes or monocytes. Distinction between the last named types of cells has been somewhat overemphasized.[28-30] We are in agreement with the belief [31,32] that they are closely related and often indistinguishable from each other. They tend to be smaller than the stem cells of the preceding group but are distinctly larger than lymphocytes, varying from 14 to 22 μ in diameter. The cytoplasm is abundant, generally eosinophilic, and its borders, though distinct, tend

tumor (1) as an immature cell of the lymphocyte series, (2) as a pluripotential cell of variously assumed potentialities of development including the formation of lymphocytes, phagocytes and of reticulum and collagen, and (3) as a relatively well differentiated cell of the monocyte or clasmatocyte series. When such variation of opinion exists it seems probable that the individual authors cited cannot be describing the same tumor; that, in fact, reticulum cell sarcoma, as the term is at present used, represents not an entity but a blanket-term covering all primary tumors of lymph nodes not otherwise classifiable. Our own observations lead us to believe that so-called reticulum cell sarcoma must be divided into two types: (1) tumors composed of relatively well differentiated wandering cells with phagocytic propensities resembling monocytes or clasmatocytes, and (2) tumors made up of highly undifferentiated, presumably pluripotential cells which we have chosen to call stem cells. The justification of such a step will, we hope, be made apparent in the descriptive sections to follow. For those who hesitate to accept such identification, this "stem cell" group may simply be regarded as consisting of those tumors of the lymphocytic series too undifferentiated to classify. With this single exception our classification does not deviate from generally accepted cytologic lines.

1. *Stem Cell Lymphoma*

There is general agreement concerning the existence in lymph nodes of an undifferentiated cell of mesodermal origin which, as a result of unknown stimuli, develops the ability to differentiate into various forms of blood cells.[16-19] A structurally indistinguishable cell is to be found throughout the remainder of the hematopoietic system. The extent of its pluripotentiality in lymph nodes and elsewhere is still unsettled and cannot be discussed in this paper. Such cells have variously been termed lymphoidocyte,[20] primitive blood cell,[21,27] hemohistioblast,[22] hemocytoblast,[23] reticular [24,25] and reticulum cell [12,13,26] and common lymphoid stem cell.[27] The last term seems to us simplest and most appropriate and will be used throughout the article.

The lymphoid stem cell is a large cell, 15 to 35 μ in diameter, with variably abundant, pale-staining, amphophilic cytoplasm possessing a poorly defined, often imperceptible outline. When closely packed, as in the center of a hyperplastic follicle, the cells may appear fused as if constituting a syncytium. Where the cells are more discrete, intercellular bridges are sometimes noted. The nucleus is large, two to four times that of a normal lymphocyte, is usually round and its border is thin but distinct. Chromatin is extremely delicate, irregularly distributed and generally lacks points of condensation. There is, however,

4. *Lymphocytic Lymphoma*

The predominating cell in this lesion is indistinguishable from a normal lymphocyte (Fig. 10). Stem cells and lymphoblasts are present in small numbers scattered indiscriminately, but usually are too infrequent to cause diagnostic difficulties. Mitotic figures are sparse and no multinucleated cells appear. Nodal architecture, including sinus and follicle structure, is characteristically obscured by the relatively uniform infiltration of small lymphocytes (Fig. 9). As in the preceding group, however, stromal revision is frequently incomplete and some irregularity remains as the result of persisting portions of uninvolved nodal tissue or of small focal collections of less mature cells. The nodal capsule is usually intact but invasion may occur. Extension into perinodal tissues simulates closely the appearance of the original nodal lesion.

Leukemia. Upon completion of both the clinical and histologic studies an attempt was made to predict the presence or absence of clinical leukemia on the basis of the nodal morphology in both this group and the lymphoblastic lymphomas. No criterion for distinction held. Nodes with apparent blood-vessel invasion were obtained from patients without leukemia, and many with pericapsular invasion or large invasive tumors simulating Kundrat's lymphosarcoma [34] were accompanied by leukemia. It was necessarily concluded that it is impossible to distinguish the leukemic from the non-leukemic lesion by means of lymph node morphology.

5. *Hodgkin's Lymphoma*

Hodgkin's disease (the eponymic terminology is still more widely accepted than any of the suggested synonyms such as malignant lymphadenoma or lymphogranuloma) constitutes the commonest and most readily recognizable lesion among the malignant lymphomas. Its range of clinical and histologic variation is so great that subdivision appears necessary and the two terms, Hodgkin's lymphoma and Hodgkin's sarcoma, have been employed to designate the two divisions which we have recognized. Of the utility of a third subdivision, Hodgkin's granuloma,[35,36] we are still unconvinced. In contrast to the preceding groups, in each of which proliferation of a single type of cell completely dominates the picture, Hodgkin's lymphoma is essentially polycellular (Fig. 11). The majority of the constituents; *i.e.*, granulocytes (usually eosinophilic but frequently neutrophilic), lymphocytes, plasma cells, clasmatocytes and fibroblasts, are the usual components of various inflammatory reactions. Other elements, however, the only elements, moreover, which can be regarded as pathognomonic and whose pres-

to be irregular in outline, suggesting ameboid propensities (Fig. 5). Phagocytic qualities are marked and, though usually limited to particles, engulfment of whole cells occurs. Nuclei are frequently eccentric in position; a few are round, more are oval and still others are reniform or even horseshoe shaped (Fig. 6). Chromatin forms a moderately fine network and nucleoli are rarely evident. The rate of growth and degree of differentiation varies over considerable limits, but in most tumors sufficient numbers of apparently mature monocytic or clasmatocytic elements are present to aid materially in identification.

In the less differentiated examples the distinction from stem cell lymphoma is difficult and in some neoplasms, which by all other criteria appear to belong in this group, the presence of multinucleated cells makes confusion with Hodgkin's sarcoma possible. Small numbers of lymphocytes are occasionally found sparsely and irregularly sprinkled, presumably evidence of exudative reaction. Reconstruction of reticulum is relatively scanty. Scattered fibrils without regular network are observed in most lesions. There is little evidence to support the contention that fibrils arise from the tumor cells.[1,9,33] A few of the specimens exhibit a coarse latticework of collagen but none is actually scirrhous in appearance.

3. Lymphoblastic Lymphoma

The predominant cell in these lesions is a lymphoblast. It is a spherical cell which, however, frequently exhibits irregularity of outline with pseudopod-like protuberances. It is larger than a mature lymphocyte, varying ordinarily within the range of 10 to 20 μ in diameter, and possesses a relatively uniform, narrow, basophilic rim of cytoplasm (Figs. 7 and 8). The nucleus is likewise larger than that of the lymphocyte, is centrally placed, round or slightly indented. The nuclear border is sharp, the chromatin rather evenly distributed and much less clumped than in the mature elements, giving the nucleus as a whole a vesicular appearance. Nucleoli are infrequently observed. As would be expected in a relatively undifferentiated tumor, mitotic figures are usually numerous.

Though the lymphoblast is always the predominant cell in specimens included in this group, stem cells are present in moderate numbers and in some cases considerable numbers of lymphocytes can be found. The nodal architecture is characteristically obscured. Many specimens exhibit a homogeneous appearance as the result of the uniformity of component cells and the even distribution of the reticulum framework. The majority, however, are somewhat irregular in appearance because of incomplete stromal revision.

Granulocytes, both eosinophilic and neutrophilic, are usually but not invariably present. They bear no constant relation to the occurrence of necrosis. Eosinophils are more common and in some cases constitute the predominant cell. Monocytes and clasmatocytes are present in variable numbers. Hodgkin's lymphoma exhibits more tendency to focal necrosis than the other types of lymphoma and in lesions showing this phenomenon phagocytes are quite numerous. In a few cases, however, in the absence of evident necrosis, phagocytic cells were so abundant that distinction from clasmatocytic lymphoma was difficult.

Collagen production is roughly proportionate to the duration of the lesion and is comparatively scanty in the early phases. This stage, however, is transient; and wavy, non-argentophilic, interlacing strands soon appear, progressing steadily by fusion of the strands until broad fibrous septa separate the foci of cellularity into islands in the scirrhous tumor (Fig. 13). Still later, if the disease is sufficiently prolonged, the entire node becomes replaced with dense fibrous tissue. Nodes of this type are found as frequently in areas not subjected to roentgen therapy as they are in regions so treated. Such a lesion is not then necessarily significant of the effect of irradiation.

6. *Hodgkin's Sarcoma*

It has been stated that Hodgkin's lymphoma may, after following a comparatively benign, prolonged course, undergo both clinical and histologic transformation into a rapidly progressive, highly malignant tumor.[35,37,38] Only a few cases in this series have exhibited histologic metamorphosis of this type (Table I), although many clinical histories have suggested that such a change has taken place. Most of the cases of Hodgkin's sarcoma have shown the characteristic morphology of this type at the outset.

Hodgkin's sarcoma retains the fundamental background of Hodgkin's lymphoma, the basic cell being the tumor stem cell or Sternberg-Reed cell. The peculiar difference is the marked preponderance of these cells over all other elements comprising the tumor (Fig. 14). Characteristically, the lesion consists of large numbers of these cells without syncytial relations, exhibiting marked variability in size and nuclear configuration (Fig. 15). Multinucleated cells predominate and mitotic figures are very numerous. Lymphocytes, plasma cells and eosinophils, though present, are minimal in numbers and fibrosis rarely proceeds beyond the early background of strandlike collagen. Densely scirrhous tumors do not occur. Monocytes and clasmatocytes are present, many of the latter attaining features simulating neoplastic change. In certain of the tumors this is so striking that distinction from undifferentiated forms of clasmatocytic lymphoma becomes difficult.

ence is universally admitted essential for diagnosis of the lesion, are not found in any inflammatory process of established etiology. They consist of stem cells, frequently indistinguishable from those of "stem cell lymphoma," which tend strongly to develop large multilobed or multinucleated forms (Fig. 12). It is important from the diagnostic point of view, moreover, to recall that both Sternberg [7] and Reed [8] noted mononucleated as well as multinucleated "giant cells" in this disease. Although their names are commonly applied to the multinucleated forms, cells with single or mirror-image double nuclei are equally characteristic of the process.

The specific cells are quite variable in size, ranging from 10 to 40 μ, or more, in diameter. The cytoplasm is abundant and its staining reactions, though usually intense, are variable, ranging from acidophilia to basophilia in different cases, though relatively constant in the same case. Single nuclei are large and round, oval, or slightly indented; they are vesicular in appearance and contain chromatin without characteristic distribution. Within the majority, single nucleoli are found which differ from those noted in the stem cell tumor in that they are not densely stained but are actually vesicular in appearance.

In the multinucleated forms the nuclear masses may show narrow connecting bridges or may be entirely discrete. In either case large nucleoli are usually evident in each nuclear mass and the nuclear masses are characteristically dissimilar in size and shape. Even where each of the many nuclei is discrete they tend to overlap and remain clustered in the center of the cell. Unipolar mitotic figures are easily found and multipolar mitoses are not unusual. Mitotic activity is unusual, however, in any of the other cells composing the lesion.

A great variety of other elements are always to be observed in varying frequency and abundance. Although general trends are apparent, no constant chronologic sequence can be made out for the appearance of each type of cell. It is necessary to remember that the disease does not run a simultaneously parallel course in each node of a given patient. Early lesions may be evident in one region while the process is advanced in another area. The approximate age of an individual lesion may be roughly estimated from the histologic appearance but not the duration of the disease in the patient.

Lymphocytes are always present. They are most abundant in the early stages and their relative numbers diminish later with progression of fibrosis or with dedifferentiation of the lesion. Plasma cells are frequently encountered although they are more evident in advanced stages. In no other lymphoma subgroup do they appear with an equal degree of frequency.

peculiarities deserve greater emphasis in a presentation of this nature.

Significant numbers of multinucleated cells do not appear, nor are there evidences of necrosis or inflammatory exudation. Invasive qualities are noted infrequently. In two cases in which focal skin involvement occurred the cutaneous lesions also manifested follicle formation.

Nodal substance intervening between follicles varies considerably with the degree of follicular contiguity. Fusion of two or more impinging follicles occurs occasionally and when these contain "germinal centers" the latter become confluent and the separating rims of small lymphocytes are lost (Fig. 17). In general, the internodular tissue consists of closely packed normal lymphocytes and the compression and obliteration of sinus spaces simulates the appearance of lymphocytic lymphoma. Such similarity is dispelled, however, by reticulum stains and by lower power examination under which the follicular structure becomes obvious.

Since isolated follicle fusion is observed, it is conceivable that, if the course were sufficiently prolonged, fusion would become quite general and the end result indistinguishable from other types of lymphoma. In eight cases in which histologic studies were possible 1 to 11 years after an initial biopsy, transformation into lymphoblastic lymphoma was observed in one case. In all of the others there was persistence of recognizable follicular structure. In these, although a few regions showed coalescence with loss of nodular structure, reticulum stains exhibited the residuum of the characteristic framework described above.

PERSISTENCE OF HISTOLOGIC TYPE

At this point the question of persistence of histologic form in malignant lymphoma in general naturally arises. In 84 cases in this series biopsies were made on two or more occasions, or specimens from both biopsy and subsequent necropsy were available. In 56 of these cases a period of at least 1 month intervened between the two specimens. The average interval in this group was 2.3 years and the range up to 15 years. As shown in Table I, the original histologic structure was maintained in 43, or 76.8 per cent, of the cases. In the remaining 23.2 per cent (13 cases) the lesion became less differentiated in appearance and would, therefore, have been placed in another lymphoma group had the original biopsy not been available. It is believed reasonable to expect this degree of dedifferentiation in any group of malignant tumors followed over an extended period of time. In this particular instance the occasional transition from one form to another is a feature lending credence to the belief that these tumors are essentially of common origin.

7. *Follicular Lymphoma*

This unusual subgroup was originally segregated as "giant lymph follicle hyperplasia" by Brill, Baehr and Rosenthal [39] but later classified as a manifestation of malignant lymphoma.[40,41] It has been implied by Ewing [11] and Jackson [36] that it constitutes an inconstant borderline group, evidently a developmental phase of variable duration ultimately becoming one of the other types of lymphoma. Callender [1] attributed a relatively high degree of malignancy to this type while Symmers at first [42] did not believe that the condition was neoplastic at all and later [43] conceded this point with considerable reservation. Although there is evidence that with a sufficiently prolonged course the structural arrangement of the follicular lymphomas may eventually approximate that of one of the other types of lymphoma, there can be little question relative to either the individuality or the ultimate malignancy of the lesion.[44]

Fundamentally, it manifests itself by complete replacement of normal lymph node architecture by multiple follicle-like nodules of varied size and approximation (Fig. 16). These are also present in regions of extranodal invasion. The structure of nodal reticulum is characteristically revised. Trabeculae are obscured. Surrounding each follicular nodule the reticulum meshwork is obviously distorted and condensed by the expanding follicle, and the normally loosely arranged network with broad, polygonal pulp spaces (Fig. 19) becomes compressed and the inter-reticular spaces markedly elongated and narrowed (Fig. 21). Such stromal rearrangement does not occur in ordinary hyperplasia of lymph nodes and may therefore be considered to be of some diagnostic value. Despite this condensation of interfollicular fibrils, actual fusion with the production of collagen does not occur. The sinuses are evidently obscured by displacement of condensed fibrillar material into them. The sparse, stretched attachments of follicles to the surrounding framework allow for separation of the follicles from surrounding tissues in the process of sectioning. This seeming "cracking off" of follicular from nodal substance is due to artefact but is sufficiently constant to serve as a differentiating criterion (Figs. 16 and 18). It is observed relatively uncommonly in other lymph node conditions.

In sections stained in the usual fashion with phloxine and methylene blue several types of follicular nodules are observed. These vary in different cases, apparently with the duration of the ailment, but are type-constant in a given specimen. Detailed description of this phenomenon has been recorded elsewhere [44] and will not be further elaborated here. Although these intrafollicular variations represent features characteristic of this form of lymphoma, the general morphologic

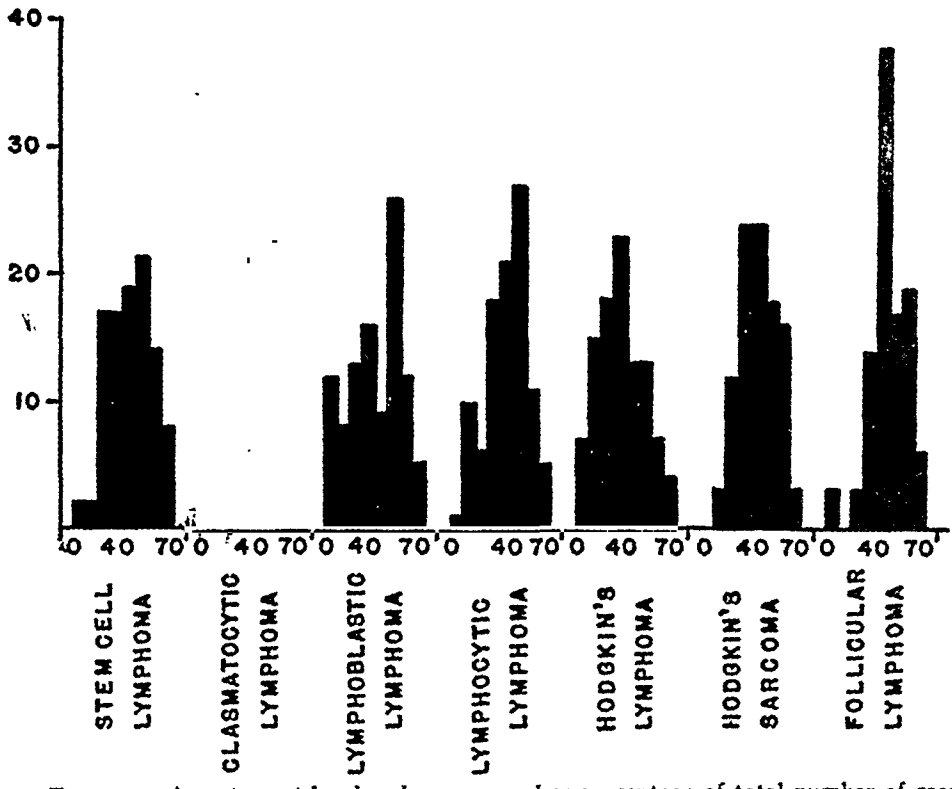
levels occurred in Hodgkin's lymphoma where both figures indicate a tendency for the disease to appear nearly a decade earlier than with any of the other subtypes. Slight deviations in the other direction above the mean level are apparent in the clasmatocytic and follicular subgroups.

TABLE II

Age at Onset (Years)

Type	Males		Females		Total	
	Average	Median	Average	Median	Average	Median
Stem cell lymphoma	47	50	43	41	46	48
Clasmatocytic lymphoma	45	43	52	55	49	49
Lymphoblastic lymphoma	44	43	37	38	41	45
Lymphocytic lymphoma	45	49	44	44	44	47
Hodgkin's lymphoma	37	30	34	34	36	34
Hodgkin's sarcoma	44	44	46	43	45	43
Follicular lymphoma	51	49	49	42	50	46

More convincing evidence of characteristic group variation is provided in Text-Figure 2 in which age distribution is charted. Although examples of each of the specific types were met in every age group, it is apparent that age distribution is more or less characteristically conditioned by histologic type. For all types except Hodgkin's lymphoma the disease most frequently became evident in the fifth and sixth



TEXT-FIGURE 2. Age at onset by decades, expressed as percentage of total number of cases in each subgroup.

Part II. Clinical Studies

If a cytologic classification of the malignant lymphomas such as has been outlined is to have practical value, it should be possible to demonstrate concomitant clinical variations in the subgroups which have been distinguished. An effort will be made to show that this is, within certain limits, the case. Obviously, in a group of diseases so kindred a considerable degree of similarity and of overlapping must

Table I

Retention of Histologic Structure

Type	Average interval between specimens	Number of cases	Unchanged	Dedifferentiation	Differentiation
Stem cell lymphoma	0.25 yrs.	2	2	..	..
Clasmatocytic lymphoma	2.3 yrs.	12	6	6	..
Lymphoblastic lymphoma	0.75 yrs.	4	2	2	..
Lymphocytic lymphoma	1.9 yrs.	9	9	..	..
Hodgkin's lymphoma	1.9 yrs.	20	16	4	..
Hodgkin's sarcoma	0.5 yrs.	1	1	..	..
Follicular lymphoma	5.0 yrs.	8	7	1	..
Total		56	43	13	0

be expected. Certain differential features, nevertheless, became apparent as the material was surveyed which, though of slight importance individually, seemed collectively to delineate a series of fairly distinct clinical pictures.

In 545 cases the clinical records were found to be sufficiently complete to be of value. These were abstracted in detail without reference to histologic classification, then arranged according to histologic group and the clinical data for each subdivision analyzed. Records were complete to the day of death for 413 patients. Of the remaining 132, 21 were in the terminal stage when last seen and were presumed to have died shortly after the last observation. Fifty patients were lost and 61 are still alive, the majority of them under observation in the tumor clinic of this hospital.

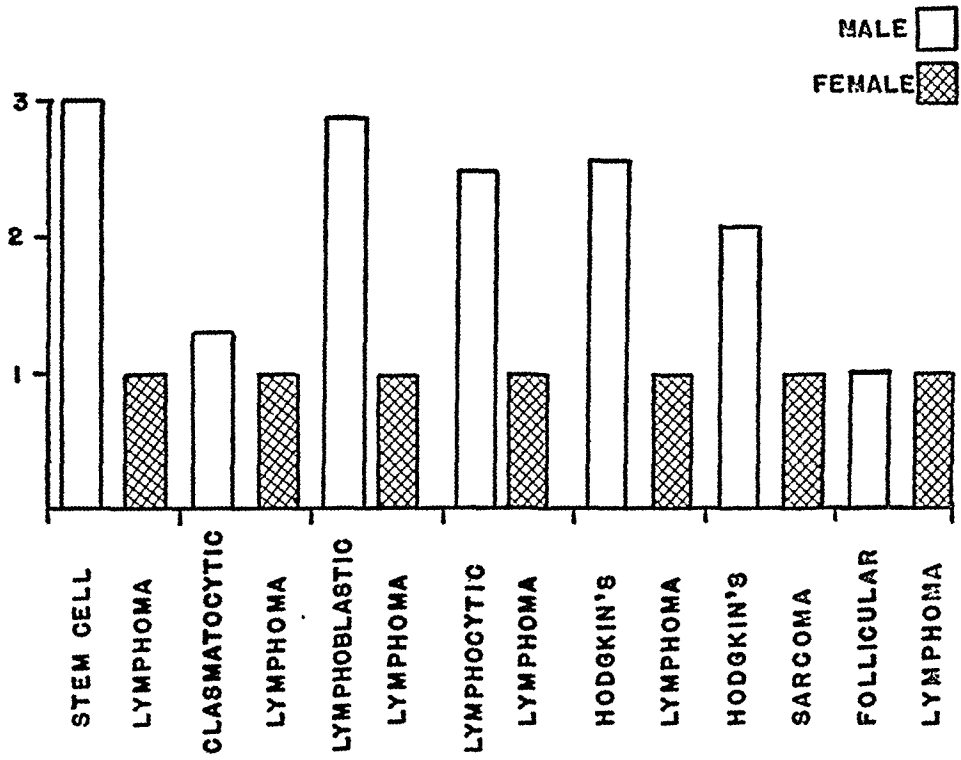
Age at Onset

The age at which the first presumably relevant symptom was noted has been recorded as the age at onset. Undoubtedly inaccurate as such a method is, the errors should approximately balance and, consequently, comparisons between the various subgroups should be valid. Figures for the average and median ages of onset of each of the subgroups appear in Table II. For the entire group the average was 42.5 years and the median 44. The most notable deviation from these

sons or contrasts, or because they have received widespread comment in the literature. In the descriptive section to follow, emphasis has been placed upon manifestations which reflect differences between the various subgroups.

Enlargement of Lymph Nodes and Spleen

Palpable, visible, or presumptive lymph node enlargement was present in well over 90 per cent of the subgroups. Among only the patients with clasmatocytic and stem cell lymphoma were there 20 per cent



TEXT-FIGURE 3. Sex incidence expressed in terms of relative proportion.

without evidence of disease of lymph nodes. Peripheral lymph nodes were enlarged most frequently in Hodgkin's, lymphocytic and follicular lymphoma and retroperitoneal nodes were outstandingly prominent in the follicular type. Mediastinal nodes were enlarged in roughly the same proportions in each group although they were noted less frequently in the clasmatocytic and stem cell lymphomas. Splenomegaly was present most frequently with lymphocytoma (56 per cent) and slightly less frequently with Hodgkin's, lymphoblastic and follicular lymphoma (34 to 46 per cent). Splenic enlargement was infrequent in Hodgkin's sarcoma and in clasmatocytic and stem cell lymphoma (14 to 23 per cent).

decades, the latter usually leading by a small margin. Ordinary Hodgkin's, in contrast, showed maximal incidence in the third and fourth decades.

Only three types of lymphoma occurred with significant frequency in the first two decades. Well in the lead in development in youth was Hodgkin's lymphoma in which 24 per cent of the cases appeared before the age of 20. Most nearly comparable was the lymphoblastic type with 18 per cent of cases in this age period. In third place with 11 per cent below 20 years of age was lymphocytic lymphoma which proved unique in showing a higher percentage of occurrence in the second decade than in either the first or third, a finding reinforced by the similar observation of Jackson.[35]

At the other end of the scale come the clasmatocytic and follicular types. Both of these were extremely infrequent in youth and relatively uncommon in the twenties and thirties as shown by the fact that only 22 and 21 per cent respectively occurred below 40 years of age. Somewhat intermediate distribution was recorded for the stem cell type and Hodgkin's sarcoma. Here, too, cases were rare before 20 years of age but enough appeared in the third and fourth decades to give incidences of 38 and 42 per cent respectively below the age of 40.

Sex Incidence

The greater frequency of malignant lymphoma in the male sex has long been recognized. For the entire series the proportion of men to women affected was 2.2:1. Hodgkin's lymphoma was the only subgroup strictly adhering to this mean. The other types, as may be seen by reference to Text-Figure 3, showed greater male predominance ranging up to 3 to 1 in the lymphocytic, lymphoblastic and stem cell groups whereas in the follicular and clasmatocytic types the frequency was approximately equal in males and females. It is possible that the lack of evident sex difference in these last two groups is dependent on the relatively advanced age at which they commonly occur.

None of the comparative variations in age incidence or duration of the disease in the two sexes recorded in Tables II and IV appears great enough to have significance unless the marked incidence of clasmatocytic lymphoma in older women (median for women 55 years against 43 years for men) should be substantiated in a larger series.

GENERAL CLINICAL MANIFESTATIONS

In Table III a large number of clinical features have been tabulated for each of the seven subgroups. They have been selected either because they appeared to offer opportunities for noteworthy compari-

Fever

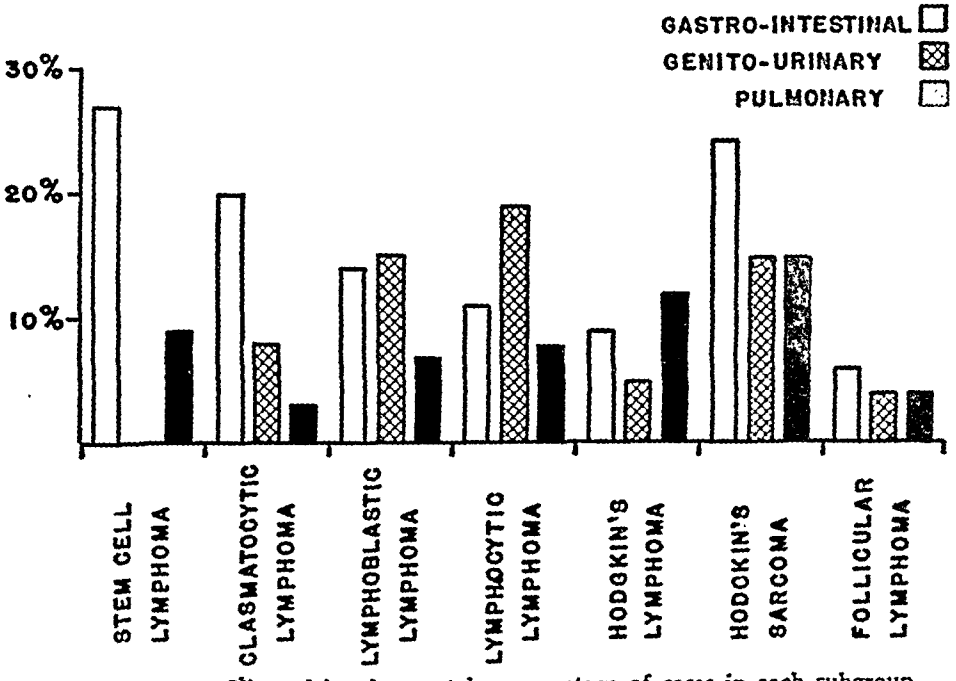
Febrile manifestations (fever of 101° F. or higher) occurred at some time during the course of the disease in cases from all the subgroups (Table III). It was rare in the follicular type (only 12 per cent), relatively uncommon (25 and 29 per cent) in the clasmatocytic and stem cell groups, appeared in 42 and 43 per cent of the lymphocytic and lymphoblastic cases, in half the Hodgkin's lymphomas and was most frequent in Hodgkin's sarcoma (59 per cent). Intermittent fever of the Pel-Ebstein type occurred with significant frequency (16 per cent) only in the two Hodgkin's types and was noted very infrequently among the others.

Cutaneous Involvement

Generalized cutaneous infiltration, scattered nodular lesions of the skin, or a combination of both of these appeared in 16 to 26 per cent of the cases in all groups except the follicular lymphomas, among which cutaneous lesions were unusual. Pruritus was recorded in cases in each group except that of follicular lymphoma. Although individuals with skin lesions were prone to suffer from this symptom, it bore no constant relationship to the presence of evident cutaneous disease and was not believed to be of diagnostic value.

Visceral Involvement

There appeared to be a significant group variation with regard to visceral predilection (Text-Figure 4). The gastro-intestinal tract was



TEXT-FIGURE 4. Visceral involvement by percentage of cases in each subgroup.

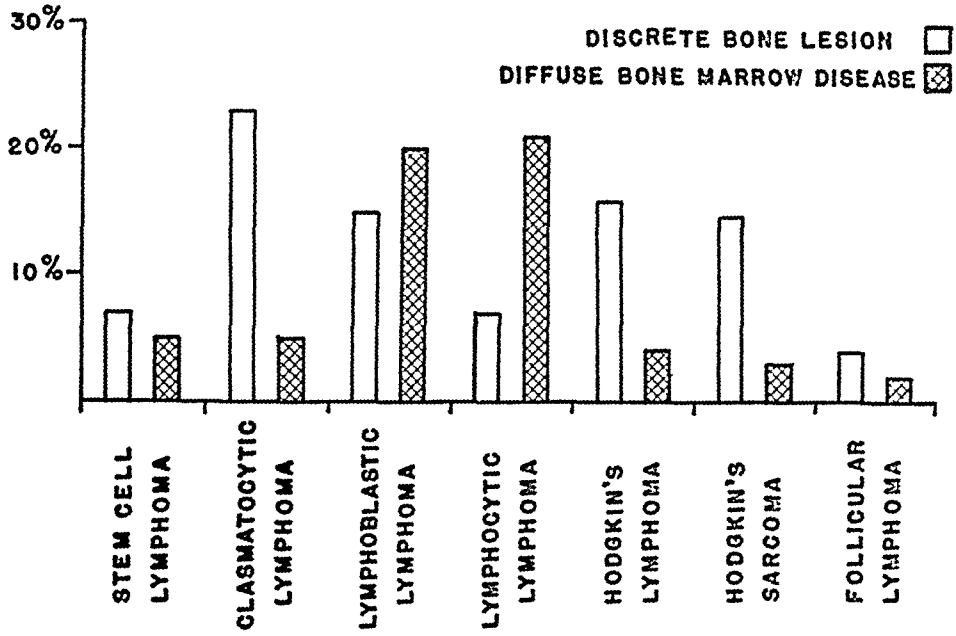
TABLE III

Distribution of Clinical Observations According to Type of Lymphoma

	All types	Stem cell	Clasmatocytic	Lymphoblastic	Lymphocytic	Hodgkin's	Hodgkin's sarcoma	Follicular
Miscellaneous data								
Number of cases	545	42	64	76	118	174	33	38
Age of onset (average)	42.5	46	49	40	44	36	45	50
Onset under 20 years	13%	4%	2%	20%	11%	22%	3%	3%
Onset under 40 years	44%	38%	23%	49%	35%	63%	39%	20%
Onset over 40 years	56%	62%	77%	51%	65%	37%	61%	80%
Average total duration (yrs.)	2.9	1.7	2.1	1.4	3.3	4.2	1.8	5.6
Mortality in initial 2 years	53%	80%	76%	80%	45%	34%	89%	13%
Five-year survivals	22%	14%	11%	3%	25%	29%	7%	53%
Ten-year survivals	4%	3%	2%	0	2%	8%	3%	16%
Radioresistant cases	9%	12%	8%	21%	3%	8%	20%	5%
Proportion, male: female	2.2	3.0	1.3	2.9	2.5	2.6	2.1	1.0
Physical examination								
Pruritus	14%	7%	11%	12%	23%	21%	18%	0
Purpura	8%	5%	1%	13%	20%	4%	3%	0
Ulcerative phenomena	24%	42%	31%	29%	23%	17%	39%	4%
Obstructive phenomena	35%	49%	49%	39%	28%	26%	33%	58%
Hydrothorax	21%	14%	17%	26%	13%	25%	30%	31%
Ascites	18%	14%	15%	15%	13%	20%	21%	37%
Fever	40%	29%	25%	42%	43%	49%	59%	12%
Intermittent fever	7%	0	3%	3%	4%	16%	15%	0
Stigmata of tuberculosis	15%	2%	17%	10%	15%	19%	15%	20%
Lymph node involvement	91%	79%	80%	93%	91%	95%	94%	100%
Retroperitoneal lymph nodes	49%	30%	40%	50%	46%	50%	48%	79%
Mediastinal lymph nodes	45%	30%	23%	45%	40%	61%	54%	37%
Splenomegaly	40%	14%	23%	46%	56%	45%	18%	34%
Gastro-intestinal involvement	13%	27%	20%	14%	11%	9%	24%	6%
Genito-urinary involvement	10%	0	8%	15%	19%	5%	15%	4%
Pulmonary involvement	9%	9%	3%	7%	8%	12%	15%	4%
Cutaneous involvement	20%	17%	16%	21%	26%	29%	24%	4%
Discrete bone involvement	13%	7%	23%	15%	7%	16%	15%	4%
Diffuse bone marrow involvement	10%	5%	5%	20%	21%	5%	3%	2%
Hematologic data								
Anemia	40%	29%	17%	54%	41%	44%	33%	29%
Leukocytosis	44%	25%	31%	52%	59%	45%	50%	15%
Thrombocytosis	18%	15%	33%	30%	2%	18%	47%	0
Thrombocytopenia	31%	14%	10%	40%	58%	27%	3%	25%
Monocytosis	21%	15%	22%	12%	9%	35%	33%	6%
Eosinophilia	8%	10%	3%	2%	5%	15%	3%	3%
Lymphocytosis	31%	13%	16%	62%	70%	14%	7%	18%
Lymphocytopenia	8%	0	2%	2%	4%	19%	13%	0
Atypical cells	27%	7%	14%	60%	33%	25%	20%	6%
Leukemia	9%	2%	5%	15%	23%	2%	3%	3%
Subleukemia	8%	0	0	23%	25%	0	0	0

frequency only among the patients with Hodgkin's sarcoma and thrombocytopenia among those with lymphocytic and lymphoblastic lymphoma. Purpura was noted more commonly in the two last-named groups.

A leukocytosis exceeding 12,000 per cmm. was observed in 50 to 60 per cent of the patients with lymphocytic and lymphoblastic lymphoma and Hodgkin's sarcoma. It was less common with Hodgkin's,



TEXT-FIGURE 5. Bone involvement by percentage of cases in each subgroup.

clasmatocytic and stem cell lymphoma, and was very unusual among the cases of follicular lymphoma. Leukopenia with white cells numbering less than 5,000 per cmm. was noted in 11 to 16 per cent of the patients suffering from lymphocytic, lymphoblastic and Hodgkin's lymphoma. It was rare in other types of the disease.

Monocytes in excess of 10 per cent were recorded in one-third of the cases of Hodgkin's lymphoma and sarcoma and one-quarter of those with clasmatocytomas, but rarely in the remainder. Markedly diminished monocyte values occurred with the lymphocytic and lymphoblastic lymphomas.

Lymphocytosis was observed in 62 to 70 per cent of the cases with lymphocytic and lymphoblastic lymphoma. Lymphocytopenia appeared in only 19 per cent of the Hodgkin's lymphomas and 13 per cent of the Hodgkin's sarcomas. The remaining groups generally exhibited normal values. Variations in the number of eosinophils were considered to be lacking in pertinent diagnostic value. Eosinophilia was not a prominent feature in Hodgkin's disease.

more often involved in patients with Hodgkin's sarcoma, stem cell and clasmatocytic lymphoma. These lesions were very unusual in follicular lymphoma. Tumor infiltration of the genito-urinary apparatus was most common among the lymphocytomas, less common in lymphoblastic lymphoma and Hodgkin's sarcoma and rare in the other types. Lymphomatous lesions of the lungs generally exhibited the histologic features of one or the other forms of Hodgkin's disease. Pulmonary foci were comparatively rare among the other subgroups.

Obstructive phenomena as the result of compression of vascular or visceral channels were particularly striking in follicular lymphoma. There was, in general, less evidence of obstruction in the clasmatocytic and stem cell lymphomas and considerably less among the remaining subgroups. Ulceration secondary to neoplastic infiltration of skin or mucous membranes occurred most frequently with stem cell lymphoma and Hodgkin's sarcoma.

Peripheral edema and ascites appeared in over one-third of the cases of follicular lymphoma and were much less common in the remaining groups. There was no differential significance in the frequency of occurrence of hydrothorax. Chylous effusion was observed in 11 per cent of the follicular lymphomas and was rarely encountered in any other type of the disease.

Bone Lesions

Isolated skeletal lesions, in many instances solitary manifestations of the disease, were recorded in one quarter of the patients with clasmatocytoma. They appeared less frequently in Hodgkin's and lymphoblastic lymphoma and Hodgkin's sarcoma and were distinctly unusual in the other types. The presence of pathologic fracture varied directly with the frequency of discrete bone lesions (Text-Figure 5).

HEMATOLOGIC OBSERVATIONS

Of the nonspecific changes in the peripheral blood, anemia was the most important. A red blood cell count of 3.5 million or less was present in one-half to one-third of the patients with lymphoblastic, Hodgkin's and lymphocytic lymphoma and Hodgkin's sarcoma in descending order of frequency. Anemia was very much less common among the cases with follicular, clasmatocytic and stem cell lymphoma. Profound anemia was recorded most often in the lymphoblastic and lymphocytic lymphomas with leukemic manifestations and in the terminal stages of Hodgkin's disease.

Platelet estimations were infrequently made and were too inaccurate for critical analysis. Thrombocytosis occurred with significant

is known relative to the mechanism of the delivery of the abnormal cells into the blood stream or concerning the abrupt changes in the blood picture so frequently observed.

The development of leukemia was of some prognostic import. Seventy-seven patients with lymphoblastic or lymphocytic lymphoma, with leukemia recorded at least once, showed an average life expectancy of about 1 year less than those without leukemic manifestations. Its presence at the onset or its development during the course generally implied a poorer prognosis, particularly if the leukemic cells were immature or bizarre in appearance and the causative lesion was of the lymphoblastic type.

"Lymphosarcoma"

Malignant lymphoma in all its varieties tends to be a generalized disease, or to become such so rapidly that it is the exceptional case which is observed in the localized stage. From clinical study it is scarcely possible to be certain that the disease is indeed localized, but at the autopsy table isolated lesions are not infrequently met where the most meticulous examination fails to reveal any evidence of generalization. Still more convincing is the experience with surgical resection of lymphomatous tumors which may be followed by survival for many years even without postoperative irradiation; results scarcely interpretable in any other light than as the extirpation of a localized neoplasm.

Localization is not a peculiarity of any cytologic type of lymphoma but is met in all. The frequency, however, with which localized tumors are encountered varies considerably with the histologic type. In 70 of the cases in our series the lesion was limited to a single area at the time of initial examination. Their distribution is recorded in Table IV. Classified according to cytologic type, localization was observed in only 3 per cent of cases of Hodgkin's sarcoma, 6 per cent of Hodgkin's lymphoma, 10 per cent of follicular lymphoma, 11 per cent of lymphocytoma and 13 per cent of the lymphoblastic form. The frequency rose sharply to 26 per cent of stem cell and 33 per cent of clasmatocytic lymphoma.

It is of interest that over half of these lesions appeared in non-lymphoid tissues, most frequently in bones and in the gastro-intestinal tract, but occasionally in areas in which even isolated lymph follicles are not found under normal circumstances (*i.e.*, the subcutaneous fat, the bladder and the cervix of the uterus). Many of these tumors grow in a frankly invasive and destructive manner and metastases may occur in many organs without diffuse involvement of the lymphoid structures or the bone marrow.

Atypical cells, "tumor cells," unidentified cells and "blast" forms appeared in the peripheral blood of almost two-thirds of the cases of lymphoblastic lymphoma, one-third of those with lymphocytoma and one-quarter of those with Hodgkin's lymphoma and sarcoma. In only 6 to 14 per cent of the remainder were such phenomena apparent.

Leukemia

Peripheral blood pictures characteristic of leukemia as commonly defined occurred at some time during the course of the disease in 48 per cent of the cases with lymphocytoma and in 38 per cent of those with lymphoblastic lymphoma. They were infrequent but were encountered among each of the remaining subgroups except the stem cell type. The leukemic pictures were susceptible to division into the various phases commonly ascribed to this disease. These consisted of: true leukemia with the white cells numbering over 30,000 associated with an absolute increase and preponderance of cells of the lymphocytic series; sub-leukemic leukemia in which the total number of white cells fell between the normal range and minimal leukemic levels; and finally, aleukemic leukemia in which the total number of white cells was well below the minimal normal level of 5,000 but in which again there was a predominance of lymphocytes or "atypical" or immature lymphoid forms.

The significance of such a classification appears dubious when a series of cases is followed over a considerable period of time. Fifty patients who exhibited some phase of leukemia during their course were selected for analysis on the basis that two or more blood studies were recorded at extended intervals during their disease. Of these, 13 patients were non-leukemic when first observed but developed leukemia later in the course, six were leukemic at the initial observation but showed normal blood pictures prior to death and two other patients were leukemic at the beginning and at the end of the disease but were non-leukemic in the interval. Forty-two per cent of this group, then, exhibited marked relapse into, or remission from, leukemia. An additional 38 per cent showed a variety of shifts among the aleukemic, subleukemic and leukemic phases without reverting to normal. In only 20 per cent of the 50 patients was the blood picture entirely constant throughout the course of the disease.

Since, as has been shown in the first portion of this study, there is no difference in the histologic background of the leukemic and the non-leukemic cases, it appears evident that the development of leukemia must be regarded as an incidental occurrence in the disease—simply an overt manifestation of the underlying process. Actually nothing

35	Lymphoblastic lymphoma	Rectum	Surg.	0.3 years	None	Dead	
36	Lymphoblastic lymphoma	Frontal bone	X-ray	2.5 years	Local	Dead	
37	Lymphoblastic lymphoma	Tonsil	X-ray	1.7 years	Local	Dead	
38	Lymphoblastic lymphoma	Cerv. l. n.	X-ray	0.4 years	Local	Dead	
39	Lymphoblastic lymphoma	Stomach	Surg., x-ray	3.2 years	Generalized	Dead	
40	Lymphoblastic lymphoma	Parotid	X-ray	1.2 years	Generalized	Alive	
41	Lymphoblastic lymphoma	Mediastinum	None	0.1 years	Local	Dead	Yes
42	Lymphoblastic lymphoma	Mediastinum	None	0.1 years	Local	Dead	Yes
43	Lymphocytic lymphoma	Nasal sinus	Surg., x-ray	7.5 years	Generalized	Dead	
44	Lymphocytic lymphoma	Testis	Surg., x-ray	0.5 years	Generalized	Dead	
45	Lymphocytic lymphoma	Nose	X-ray	2.5 years	Generalized	Dead	Yes
46	Lymphocytic lymphoma	Parotid	None	1.0 years	Local	Dead	Yes
47	Lymphocytic lymphoma	Tonsil	Surg., x-ray	9.0 years	Generalized	Dead	Yes
48	Lymphocytic lymphoma	Cerv. l. n.	Surg., x-ray	3.8 years	Local	Lost	
49	Lymphocytic lymphoma	Prostate	X-ray	4.0 years	Local	Lost	
50	Lymphocytic lymphoma	Stomach	Surg., x-ray	7.0 years	None	Alive	
51	Lymphocytic lymphoma	Femur	Surg., x-ray	8.0 years	Local	Alive	
52	Lymphocytic lymphoma	Dura (cord)	X-ray	0.7 years	Local	Dead	
53	Lymphocytic lymphoma	Intestine	Surg., x-ray	2.4 years	Generalized	Dead	
54	Lymphocytic lymphoma	Mediastinum	None	?	Local	Dead	Yes
55	Lymphocytic lymphoma	Bladder	None	0.3 years	Local	Dead	Yes
56	Hodgkin's lymphoma	Abdominal l. n.	None	1.5 years	Local	Dead	
57	Hodgkin's lymphoma	Back	X-ray	5.5 years	Generalized	Dead	
58	Hodgkin's lymphoma	Cerv. l. n.	Surg., x-ray	8.6 years	Generalized	Dead	
59	Hodgkin's lymphoma	Abdominal l. n.	Surg.	0.6 years	None	Dead	
60	Hodgkin's lymphoma	Parotid	X-ray	9.0 years	Generalized	Dead	Yes
61	Hodgkin's lymphoma	Cerv. l. n.	X-ray	5.9 years	Generalized	Dead	
62	Hodgkin's lymphoma	Abdominal l. n.	X-ray	3.3 years	Generalized	Dead	Yes
63	Hodgkin's lymphoma	Cerv. l. n.	X-ray	8.9 years	None	Alive	
64	Hodgkin's lymphoma	Mediastinum	None	0.2 years	Local	Dead	Yes
65	Hodgkin's lymphoma	Ing. l. n.	None	0.7 years	Local	Dead	Yes
66	Hodgkin's sarcoma	Axillary l. n.	X-ray	1.5 years	Generalized	Lost	
67	Follicular lymphoma	Abdominal l. n.	X-ray	5.5 years	Local	Lost	
68	Follicular lymphoma	Ing. l. n.	Surg.	6.5 years	None	Dead	Yes
69	Follicular lymphoma	Abdom. l. n.	X-ray	2.2 years	Generalized	Dead	
70	Follicular lymphoma	Ing. l. n.	Surg., x-ray	5.5 years	None	Alive	

TABLE IV

Initially Localized Malignant Lymphoma (Lymphosarcoma; Kundrat)

No.	Type	Location	Treatment	Duration	Ultimate distribution	Result	Autopsy
1	Stem cell lymphoma	Mediastinum	X-ray	1.6 years	Local	Dead	
2	Stem cell lymphoma	Cerv. l. n.	Surg., x-ray	10.2 years	Generalized	Dead	
3	Stem cell lymphoma	Stomach	Surg.	0.3 years	None	Dead	
4	Stem cell lymphoma	Stomach	Surg.	2.0 years	None	Dead	
5	Stem cell lymphoma	Thigh	Surg., x-ray	15.7 years	Generalized	Dead	
6	Stem cell lymphoma	Abdom. l. n.	X-ray	1.2 years	Generalized	Dead	
7	Stem cell lymphoma	Dura (cord)	Surg., x-ray	2.2 years	Generalized	Lost	
8	Stem cell lymphoma	Cecum	None	0.4 years	Local	Dead	Yes
9	Stem cell lymphoma	Eyelid	Surg., x-ray	1.9 years	Generalized	Dead	
10	Stem cell lymphoma	Stomach	Surg.	3.0 years	Local	Dead	Yes
11	Stem cell lymphoma	Stomach	Surg., x-ray	0.8 years	Local	Dead	
12	Clasmatocytic lymphoma	Abdom. l. n.	Surg., x-ray	0.5 years	Generalized	Dead	Yes
13	Clasmatocytic lymphoma	Stomach	Surg.	0.3 years	None	Dead	
14	Clasmatocytic lymphoma	Tonsil	X-ray	1.1 years	Generalized	Dead	
15	Clasmatocytic lymphoma	Thyroid	Surg., x-ray	1.3 years	Generalized	Lost	
16	Clasmatocytic lymphoma	Abdominal l. n.	X-ray	1.1 years	Local	Dead	
17	Clasmatocytic lymphoma	Humerus	Surg.	15.9 years	Local	Alive	
18	Clasmatocytic lymphoma	Stomach	Surg., x-ray	7.2 years	None	Alive	
19	Clasmatocytic lymphoma	Dura (cord)	X-ray	0.4 years	Local	Dead	
20	Clasmatocytic lymphoma	Thyroid	Surg., x-ray	3.3 years	Generalized	Dead	Yes
21	Clasmatocytic lymphoma	Stomach	Surg., x-ray	4.9 years	Generalized	Dead	
22	Clasmatocytic lymphoma	Stomach	None	1.0 years	Local	Dead	Yes
23	Clasmatocytic lymphoma	Tibia	Surg.	7.5 years	None	Alive	
24	Clasmatocytic lymphoma	Clavicle	X-ray	4.8 years	Generalized	Dead	
25	Clasmatocytic lymphoma	Femur	Surg., x-ray	4.7 years	None	Alive	
26	Clasmatocytic lymphoma	Stomach	Surg.	5.5 years	None	Alive	
27	Clasmatocytic lymphoma	Thyroid	Surg., x-ray	1.6 years	Local	Dead	
28	Clasmatocytic lymphoma	Femur	X-ray	3.7 years	None	Alive	
29	Clasmatocytic lymphoma	Nasal sinus	X-ray	2.6 years	None	Alive	
30	Clasmatocytic lymphoma	Sacrum	X-ray	1.4 years	Generalized	Dead	Yes
31	Clasmatocytic lymphoma	Ing. l. n.	X-ray	4.1 years	None	Alive	
32	Clasmatocytic lymphoma	Ileum	None	0.3 years	Local	Dead	Yes
33	Lymphoblastic lymphoma	Abdominal l. n.	Surg., x-ray	1.1 years	Local	Dead	
34	Lymphoblastic lymphoma	Jejunum	X-ray	2.3 years	Generalized	Dead	

independent foci of multicentric origin cannot be stated with any degree of certainty. It is possible that any one or all of these means may be operative.

Duration of the Disease

In a group of diseases which are generally regarded as inevitably fatal, the most practical function of a classification is to aid prognosis. Review of the literature reveals that authors have accepted an extremely discouraging viewpoint regarding sufferers from malignant lymphoma, which is, to a considerable extent, borne out by the median figure of 2.0 years for our entire series. On the other hand, 116 patients, or about 20 per cent, have lived beyond a 5-year period and nearly 10 per cent have survived 8 or more years. It is therefore of importance to determine if consistent variations in survival periods can be correlated with histologic structure.

In surveying our figures it was at once apparent that since many of our patients were still alive, including many of the cases of notably long survival, a false impression would be created if these were excluded. It was therefore decided, somewhat arbitrarily, to include all living cases of 3 or more years' duration. Though such a procedure robs the figures of absolute value—which could only be obtained by the impractical procedure of retiring to a perspective of at least 10 years—it has been uniformly applied and therefore does not affect the validity of the comparative results. Average and median figures for the various subgroups appear in Table V. Inspection at once reveals that four types (lymphoblastic, stem cell and clasmatocytic lymphomas and

TABLE V

Total Duration (Years)

Type	Males		Females		Total	
	Average	Median	Average	Median	Average	Median
Stem cell lymphoma	1.4	1.1	2.5	1.3	1.7	1.1
Clasmatocytic lymphoma	2.3	1.0	1.9	1.2	2.1	1.1
Lymphoblastic lymphoma	1.4	0.5	1.4	0.6	1.4	0.6
Lymphocytic lymphoma	3.4	3.0	3.0	1.9	3.3	2.4
Hodgkin's lymphoma	4.3	3.1	3.7	3.8	4.2	3.2
Hodgkin's sarcoma	1.9	0.9	1.7	0.9	1.8	0.9
Follicular lymphoma	4.6	4.0	6.8	5.6	5.6	5.0

Hodgkin's sarcoma) offer relatively poor prognoses with medians from 0.6 to 1.1 years. The remaining three (lymphocytic, Hodgkin's and follicular lymphomas) tend to run comparatively slower courses with medians of 2.4, 3.2 and 5.0 years respectively.

Early involvement of a vital organ may lead to rapid death even with the relatively benign varieties. Conversely, in even the most

Observations of this sort have led to the concept of a special form of malignant lymphoma termed by Kundrat [34] "lymphosarcoma" and currently defined [45] as an initially circumscribed lymphoid neoplasm which breaks through its confines and invades neighboring structures by way of lymphatics. Many authors [46-49] believe such a segregation is artificial and merely represents a phase in the course of the disease. The difficulty of maintaining the concept becomes obvious in surveying a large group of cases since "sarcomatous" tumors may even appear in cases of otherwise typical leukemia. Indeed, leukemic blood pictures may develop in cases starting as apparently typical lymphosarcoma, and the confusion is hardly alleviated by the use of terms such as "leukosarcoma" [50] to describe these transitional forms. The absence of detectable bone-marrow involvement cannot be used to rule out leukemia since cases of both lymphatic and myelogenous leukemia have been observed in which marrow replacement by leukemic cells has not occurred.[4, 31, 51] It has been claimed that the type of cell appearing in the blood in leukosarcoma is distinctive and does not resemble that noted in other forms of lymphatic leukemia. This has not been our experience. We have found the character of the circulating cell to be identical with that composing the organic process from which it has arisen.

Of the 70 cases referred to above in which a single localized lesion was present at the outset, 27 ultimately became generalized in character and indistinguishable from other forms of malignant lymphoma. Among the remaining 43 cases, all of which were followed to the time of death or for a period of at least 1 year, 27 showed persistent localization of the process. Twenty-two of these died and only 11 were autopsied so that the evidence of failure of dissemination was purely clinical in 60 per cent. Furthermore, the duration of the disease had been only one-half as long as that in the 27 who developed the generalized condition (2.2 and 4.1 years).

In addition to these 54 cases, 16 showed no evidence of persistent tumor at all. Twelve of these had been subjected to radical surgical excision and 5 died immediately postoperatively. Ten of the 16 were still alive and an autopsy had been made on only 1 of the others. The evidence here also was therefore overwhelmingly clinical and unconfirmed by postmortem investigation.

It seems reasonable to conclude that the condition termed "lymphosarcoma" represents a transient clinical phase and in most instances might be expected to progress into a generalized process if the patient did not succumb at too early a period. Whether or not the dissemination when it does occur is the result of direct extension, metastasis or

belied the gloomy outlook predicated by the histologic appearance of the underlying lesion. Cases in which cutaneous, osseous or visceral lesions were secondary phenomena in the course of a generalized process obviously do not belong in this category. Among the outstanding survivals referred to, of which there were 21, 17 showed lesions primary in the regions noted above and the remaining 4 were indistinguishable from the ordinary type of ultimately generalized disease. The longest duration recorded in this series was 23 years, the patient being still alive 21 years since an initial biopsy and 5 years after another, both of which showed classical Hodgkin's lymphoma.

In several of the subgroups the duration was apparently influenced by the age at the onset of the disease. With Hodgkin's lymphoma the survival period was much shorter in those patients in whom the disease was initiated after the age of 50 years. Among the lymphocytomas the life expectancy was better during the middle decades than it was at either extreme of life and it was particularly poor in those few cases occurring in youth. Follicular lymphoma rarely occurred in the young and uniformly offered a relatively favorable prognosis as to period of survival. The four malignant types were relatively uninfluenced by age.

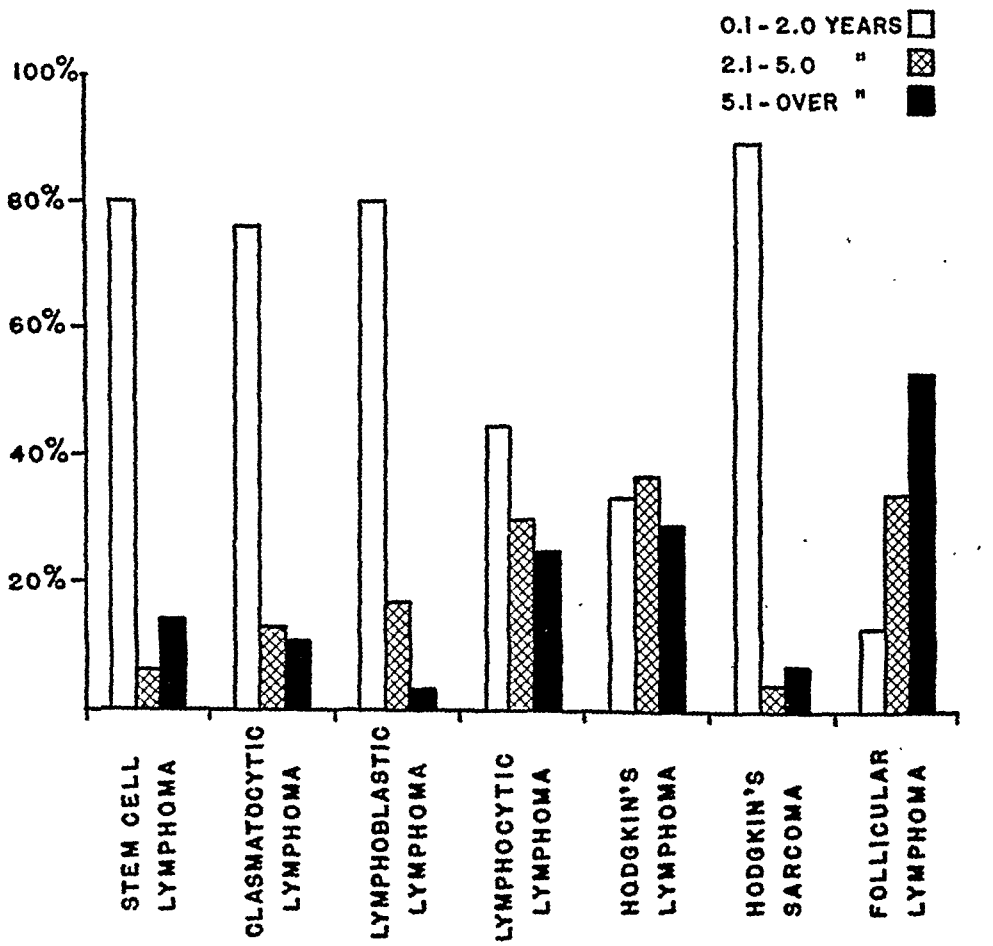
THERAPY

Roentgen therapy has been the only universally accepted means of combating the malignant lymphomas.[45,52] Indeed, lymphomatous lesions are, for the most part, so strikingly susceptible to this type of irradiation that radiosensitivity has been utilized as a diagnostic criterion for tumors not accessible for histologic study.[53] Despite this common characteristic, individual cases may fail to exhibit any favorable response to such treatment. Such failure has appeared in our experience very rarely among the Hodgkin's, follicular, lymphocytic and clasmatocytic lymphomas, although the last type occasionally required something more than the customary "lymphoma" dosage (600 r.) of deep x-ray therapy to produce subsidence of a lesion. Among the Hodgkin's sarcomas and the lymphoblastic and stem cell lymphomas, however, 20, 21 and 12 per cent, respectively, failed to show any evidence of improvement following irradiation.

It is generally agreed that the benefits of radiotherapy in the malignant lymphomas are but transitory. Moreover, it has even been claimed that although symptomatic relief might be expected, actual prolongation of the course of the disease was unusual.[54,55] Experience in the clinic leaves the observer with the impression that in many instances roentgen therapy has snatched a patient from immediately impending

malignant types, occasional instances of notably long survival (8 or more years) are met. These are least frequent in lymphoblastic lymphoma and Hodgkin's sarcoma; they occur with enough frequency in the stem cell and clasmatocytic types to raise the average duration above the median and they are most frequent in the three relatively benign varieties. It is noteworthy that 25 per cent of the cases of lymphocytic, 29 per cent of those with Hodgkin's and 53 per cent of those with follicular lymphoma lived 5 or more years after the clinical onset. On the other hand, only 3 per cent of the lymphoblastomas, 7 per cent of the Hodgkin's sarcomas, 11 per cent of the clasmatocytomas and 14 per cent of the stem cell lymphomas have survived this period (Text-Fig. 6).

Analyses of the cases with the more malignant forms of the disease which had unexpectedly prolonged courses demonstrated that group prognosis apparently was altered by several factors. Patients with primary diffuse skin disease (mycosis fungoides) or single primary lesions of a bone or a viscus (intestine, stomach, thyroid) frequently



TEXT-FIGURE 6. Total duration expressed as percentage of cases in each subgroup.

The number of individuals receiving radical surgical treatment was admittedly too small and the operative mortality obviously too high to allow unqualified recommendation of such a therapeutic measure. The selection of cases, however, was quite limited and the surgical approach in many was unduly delayed. Considering all of these factors we believe that among the malignant lymphomas there are certain cases with evidence of a single localized lesion, generally, though not always, affecting a bone or a viscus, which may be more successfully treated by radical surgery than by other means.

Summary and Conclusions

A cytologic classification of the malignant lymphomas has been presented and its advantages pointed out in a clinico-pathologic survey of 545 cases (73 cases of the 618 studied histologically had inadequate clinical data). It has been shown, by multiple examinations at significant time intervals, that the cytologic type is remarkably constant, although a few cases show a progressive failure of differentiation as the disease progresses. In contrast, in a classification based largely on distribution, such features as the presence or absence of leukemia, generalization versus localization and "sarcomatous" growth are considered important. These have been shown to be inconstant and changeable, thereby requiring variation in classification from time to time in order to fit the stage of the disease.

It was found that the vast majority of the 618 cases from which histologic material was available could be readily divided into the following seven categories: stem cell lymphoma, clasmatocytic lymphoma, lymphoblastic lymphoma, lymphocytic lymphoma, Hodgkin's lymphoma, Hodgkin's sarcoma and follicular lymphoma. This differs from widely accepted classifications primarily in the subdivision into two types of what has generally been grouped under the heading, reticulum cell sarcoma: one in which the cells are highly undifferentiated and resemble lymphoid stem cells, for which we have proposed the name stem cell lymphoma; and a second in which the cells show recognizable features of differentiation in the direction of tissue phagocytes, which we have accordingly termed clasmatocytic lymphoma. It has also proved useful to divide the tumors showing clear evidence of belonging to the lymphocyte series of cells into lymphoblastic and lymphocytic types depending upon whether the immature or mature cells predominate. Hodgkin's disease, too, has appeared divisible into lymphomatous and sarcomatous types. Follicular lymphoma has been shown to be a form of malignant lymphoma and not, as has been claimed, merely an inflammatory process.

In Part II the value of this classification has been put to the test of

death and prolonged his life by months and even years. Acceptable statistical evidence, however, that the average duration of life is prolonged is extremely difficult to accumulate. In a group of diseases where x-ray treatment has become almost automatic once the diagnosis has been established, the collection of an adequate control series is almost impossible. Out of our entire series only 76 patients were found to whom no x-ray treatment had been administered. Most of these were not seen until they were in the terminal stages of their disease, at which time therapy of this type was deemed inadvisable. A few died as the result of surgical intervention, some refused treatment and others were not treated because of failure to recognize the nature of the disease. It is interesting to note that of these 76 patients only 4 survived beyond the average duration established for their respective subgroups. The average total duration of their various illnesses was 0.8 years in contradistinction to the average duration for the entire series of 2.9 years. The two series are by no means strictly comparable since a significant degree of selection had been brought into play.

Comparison of the survival periods among the patients observed and treated between 1917 and 1926 and those seen between 1927 and 1936 showed no noteworthy difference. No improvement in results corresponding to the obviously increased efficiency of radiotherapy in the treatment of carcinoma during the same interval could be discerned.

Surgery

Surgery as a therapeutic measure in malignant lymphoma has been generally interdicted, presumably on the basis that the disease is essentially systemic.[56] In this connection it was noted that among the 135 autopsied cases in this series, 10 per cent showed localized lesions, apparently available to surgical excision and certainly not systemic in character. No evidence of the disease was found elsewhere.

In the entire series radical surgery was attempted in 77 cases, generally in ignorance of the histologic character of the lesion. In 44 cases the disease had extended beyond the limits of eradicability at the time of operation. Of the remaining 33 patients, 10 died as the immediate result of the surgical procedure. Twenty-three survivors, 12 of whom were among those listed above as exhibiting unexpectedly prolonged courses, lived or are living, an average of 7.0 years after the onset of their disease. Ten have survived an average of 6.6 years after operation without evidence of recurrence and the remaining 13 continued for an average of 5.7 years postoperatively before any evidence of recurrence appeared. Of those surviving without recurrence, one died of other causes 4.5 years after operation and at necropsy no evidence of residual tumor was found.

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clinical correlation and, although considerable overlapping was observed as would be expected in so closely related a group of diseases, sufficiently constant differences were found in the age of onset, duration of the disease, maximal frequency of involvement of various organs and tissues, tendency to localization or generalization, the development of leukemia and the degree of radiosensitivity to delineate a series of recognizably different clinical syndromes.

The following conclusions appear justified on the basis of the recorded clinical and pathologic observations:

Prognostic implications may be guided to a surprising degree by the histologic character of the lesion. However, unexpectedly prolonged survival periods have been encountered, on the one hand, in cases in which the initial lesion appeared in the skin, bone or viscera and, on the other hand, in those in which the primary lesion was sufficiently circumscribed to be susceptible to surgical extirpation. Five-year survivals have been encountered in all groups but have ranged from 3 per cent of patients with lymphoblastic lymphoma to 53 per cent of those with follicular lymphoma.

As a general rule small doses of roentgen irradiation have a favorable effect upon patients with malignant lymphoma and there is evidence to show that such therapy does prolong life. Patients with clasmatocytic or stem cell lymphoma require somewhat higher dosages to produce beneficial effects and in a few instances, notably in lymphoblastic lymphoma and Hodgkin's sarcoma, irradiation fails to produce any improvement at all.

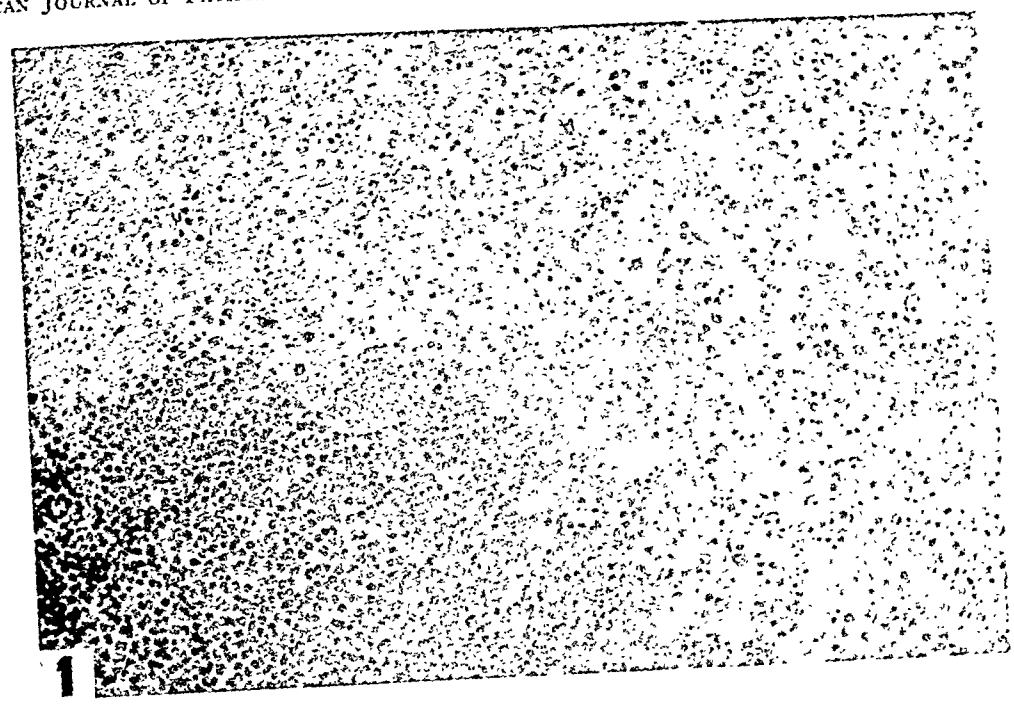
The presence or development of leukemia cannot be predicted on the basis of any constant morphologic criterion. In fact, very often the blood picture itself is inconstant and varies from time to time during the course of the disease. It seems more reasonable to consider lymphatic leukemia simply as a manifestation of an underlying lymphomatous process. Along the same lines, in the interests of clarity, it would seem judicious to discard such terms as "lymphosarcoma" (Kundrat) and "leukosarcoma" since these, too, appear merely to represent transient phases of malignant lymphoma and do not constitute disease entities.

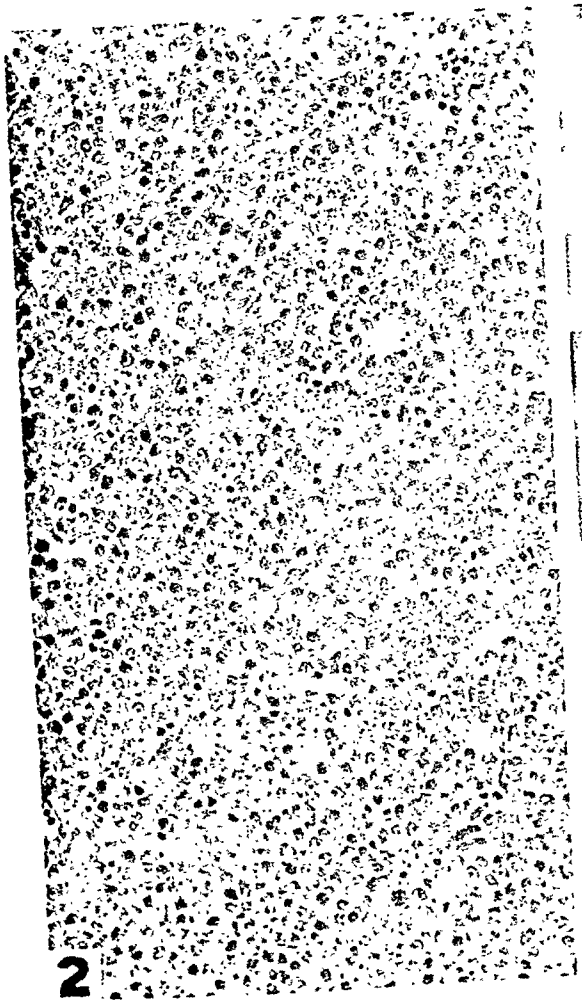
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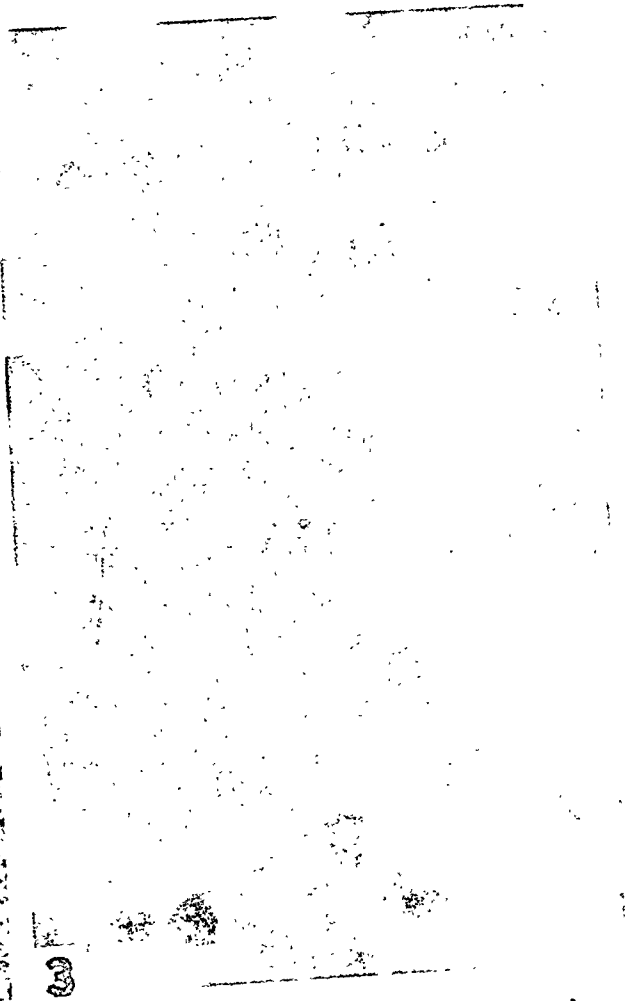
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Gall and Mallory

Malignant Lymphoma

DESCRIPTION OF PLATES

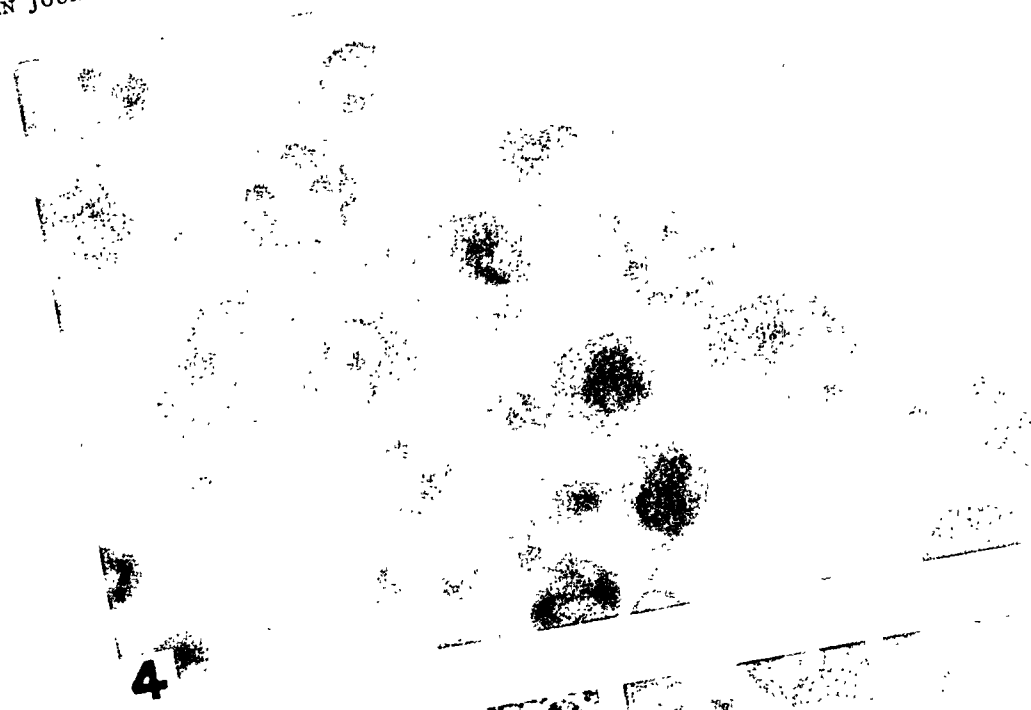
PLATE 62

FIG. 1. (Lymph node). Stem cell lymphoma, syncytial type. The dark staining area in the left lower corner of the photomicrograph represents residual normal lymphocytes in the process of being displaced by invading tumor. The infiltrating syncytial mass produces the lighter staining region in which large, clear nuclei with prominent nucleoli may be seen. Cell boundaries cannot be distinguished. × 200.

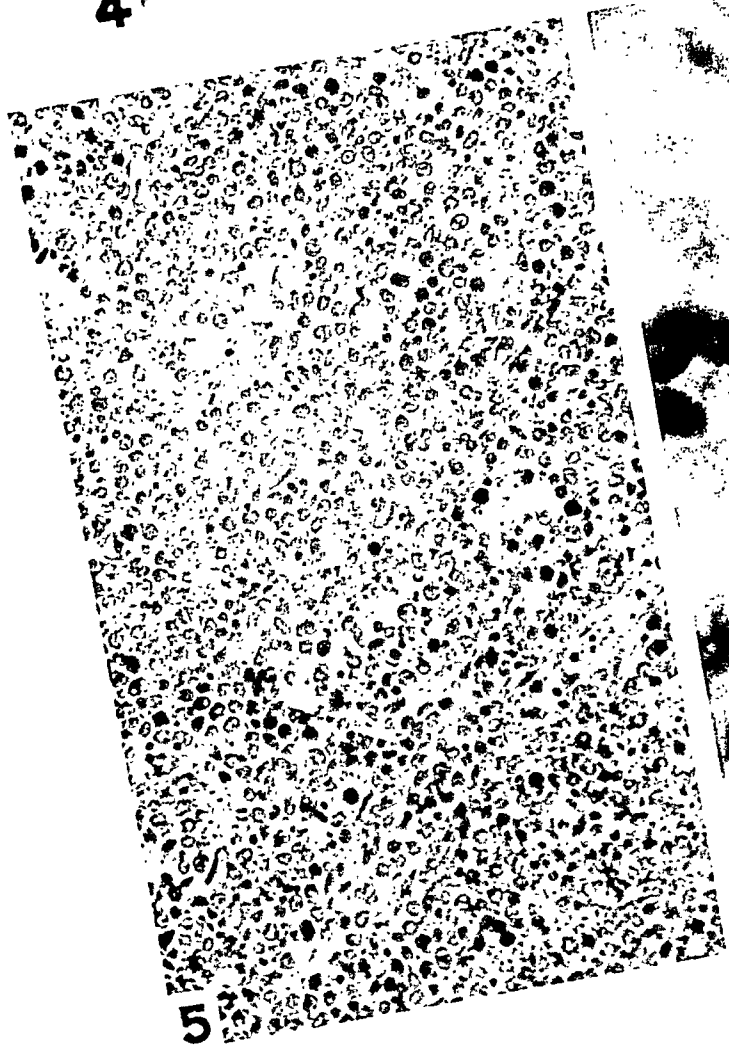
FIG. 2. (Lymph node). Stem cell lymphoma in which the cells have become discrete and intercellular connections minimized. Nuclear characteristics are unchanged from those noted in the syncytial type. × 200.

FIG. 3. (Lymph node). Stem cell lymphoma, syncytial type. The nuclei are large, clear and contain prominent, densely staining nucleoli. All cytoplasmic substance is fused and there are no cell boundaries apparent. × 1000.

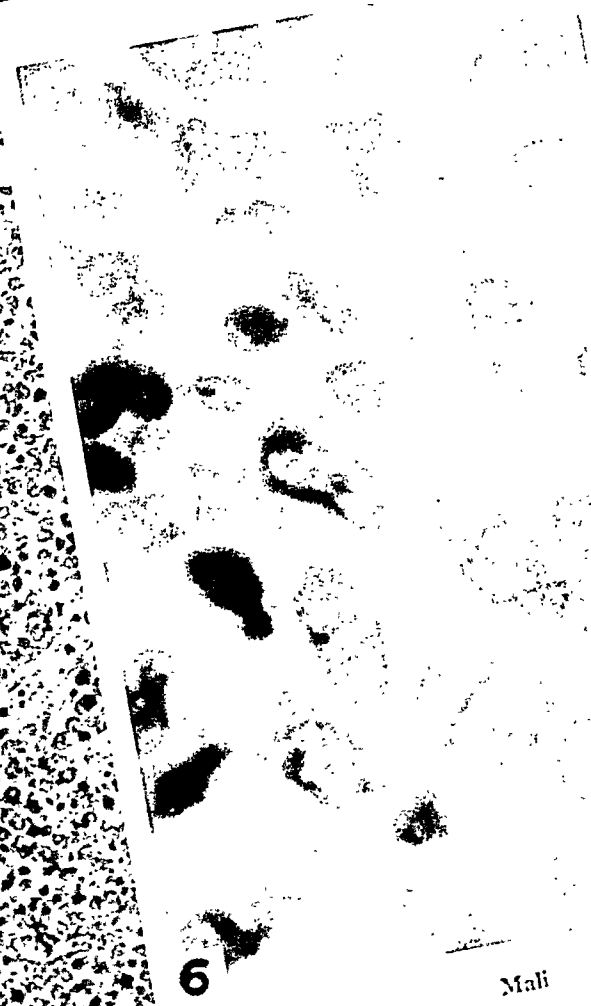
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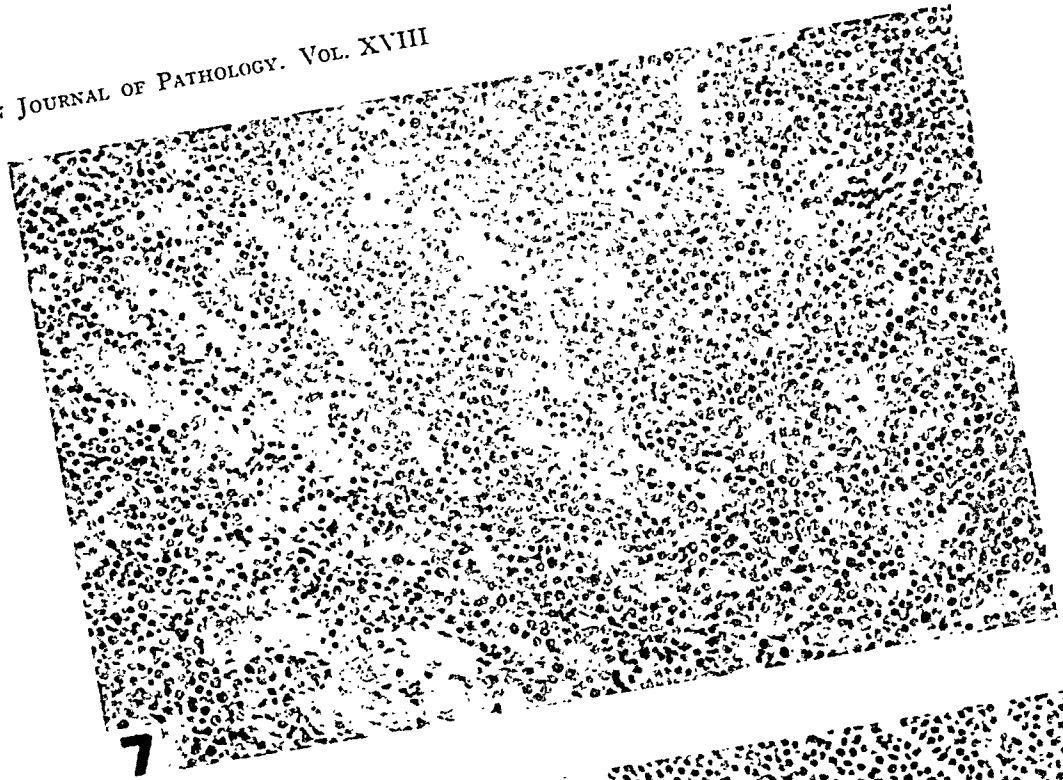
PLATE 63

FIG. 4. (Lymph node). Stem cell lymphoma. Nuclear characteristics are essentially similar to those noted in Figure 3. Although intercellular connections may be seen, cellular identity is now evident. There are a few dark staining lymphocytes present in this section. × 1000.

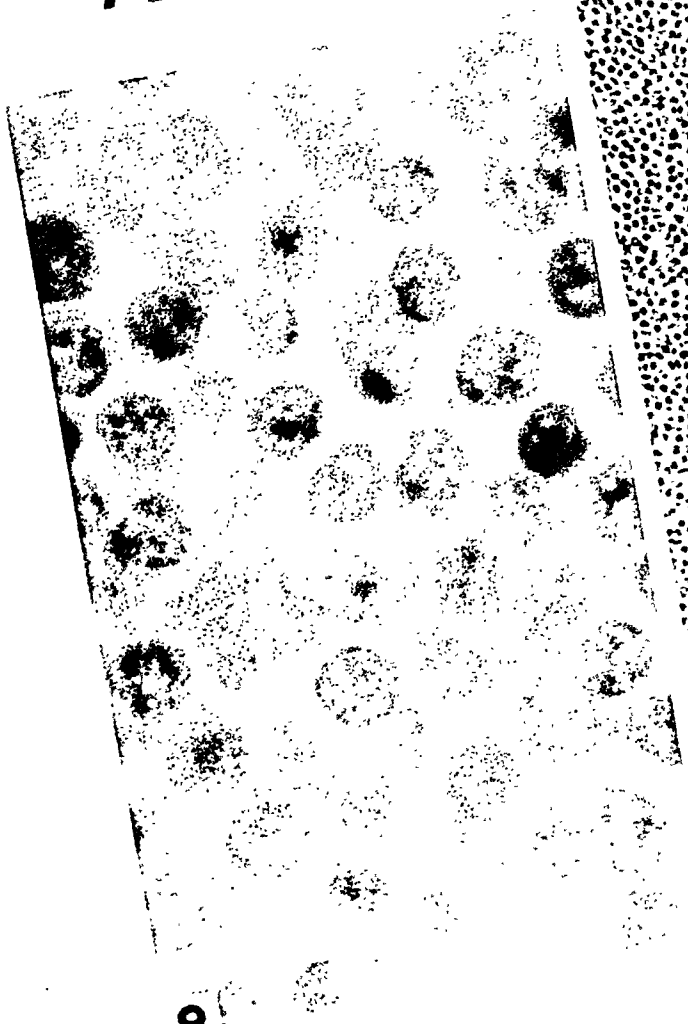
FIG. 5. (Lymph node). Clasmatocytic lymphoma. Cells similar in size to those noted in stem cell lymphoma comprise this lesion. Cytoplasm is abundant, distinctly outlined, and nuclei exhibit a marked degree of variation in configuration. × 200.

FIG. 6. (Lymph node). Clasmatocytic lymphoma. Characteristic cells show an abundant, clearly delimited cytoplasm which occasionally contains small vacuoles. Nuclei are eccentric, contain a reticulated chromatin and are reniform in shape. A stem cell is present in the right lower corner and there are several lymphocytes scattered throughout the field. × 1000.

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Malignant Lymphoma

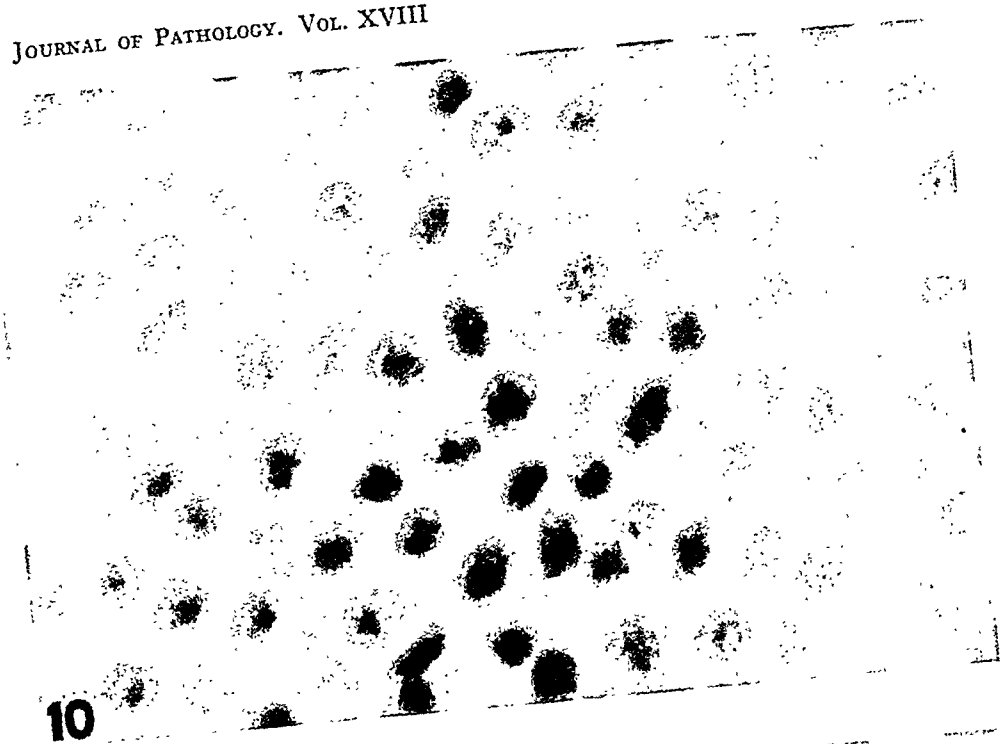
PLATE 64

FIG. 7. (Lymph node). Lymphoblastic lymphoma with revision of nodal architecture by closely packed lymphoblasts. × 200.

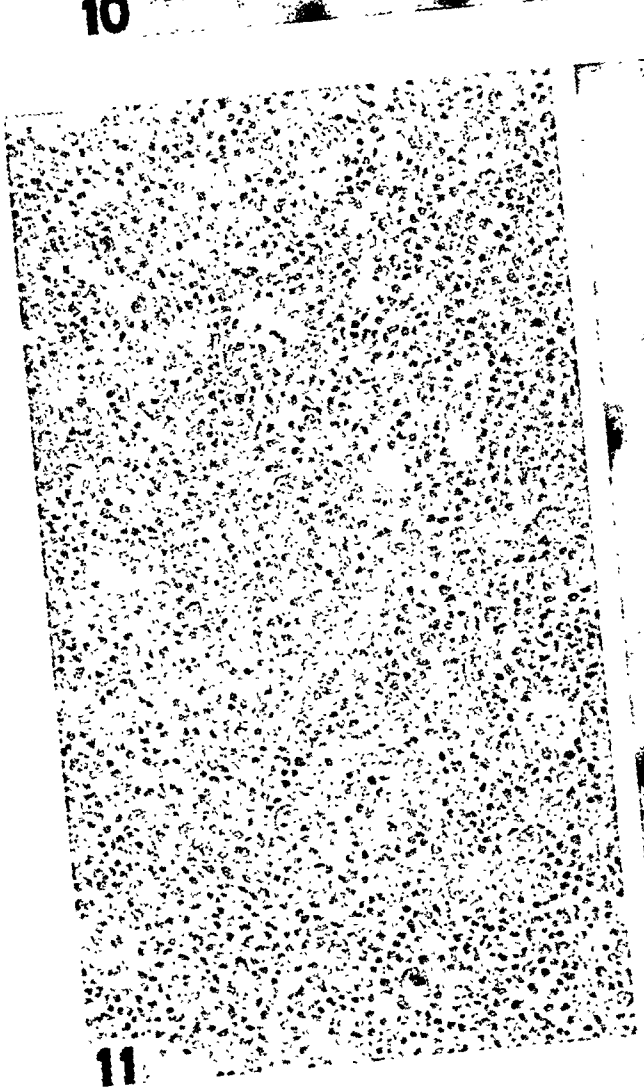
FIG. 8. (Lymph node). Lymphoblastic lymphoma; predominant cells exhibit scanty cytoplasm with larger and paler nuclei than those observed in the lymphocytic type. Chromatin is less prominent and more diffusely distributed. × 1000.

FIG. 9. (Lymph node). Lymphocytic lymphoma with replacement of normal nodal structure by uniformly distributed, small lymphocytes. × 200.

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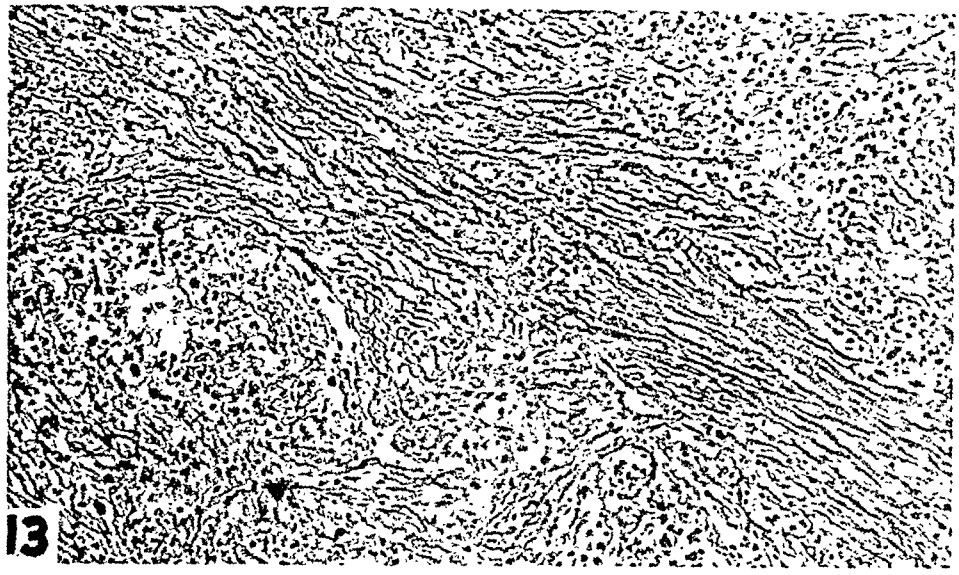
Malignant Lymphoma

Plate 65

Fig. 10. (Lymph node). Lymphocytic lymphoma showing predominance of small lymphocytes with dark staining, heavily chromatinized nuclei. An occasional, large, pale nucleus of a less mature cell may be seen. × 1000.

Fig. 11. (Lymph node). Hodgkin's lymphoma. Characteristic multinucleated giant cells are obvious. There is also a marked degree of polycellularity. × 200.

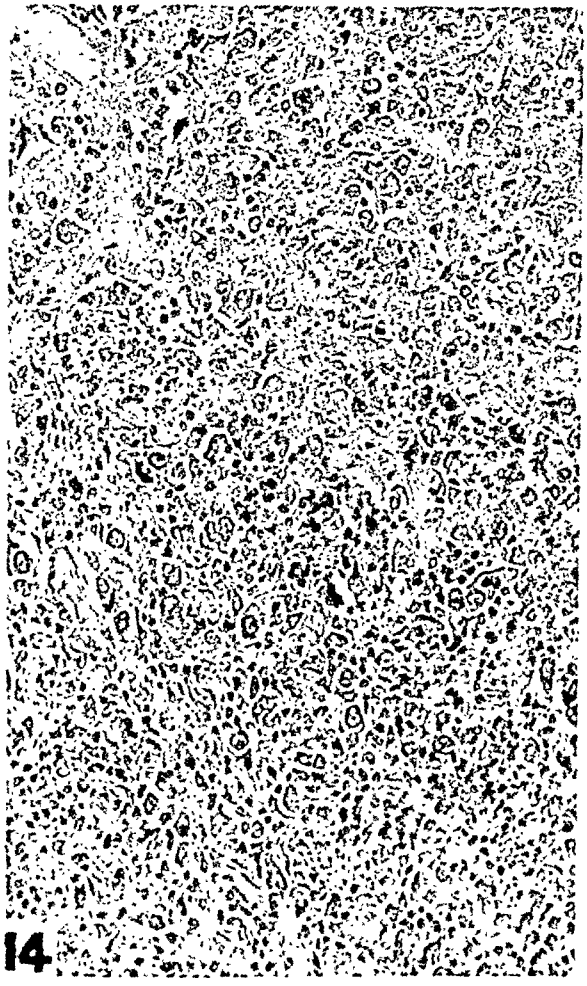
Fig. 12. (Lymph node). Hodgkin's lymphoma. In the lower portion of the photomicrograph there is a Sternberg-Reed giant cell. Scattered throughout the area may be seen lymphocytes, monocytes, fibroblasts and an occasional granulocyte. × 1000.



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...ells with bizarre, irregular ... recognizable elements are





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Malignant Lymphoma

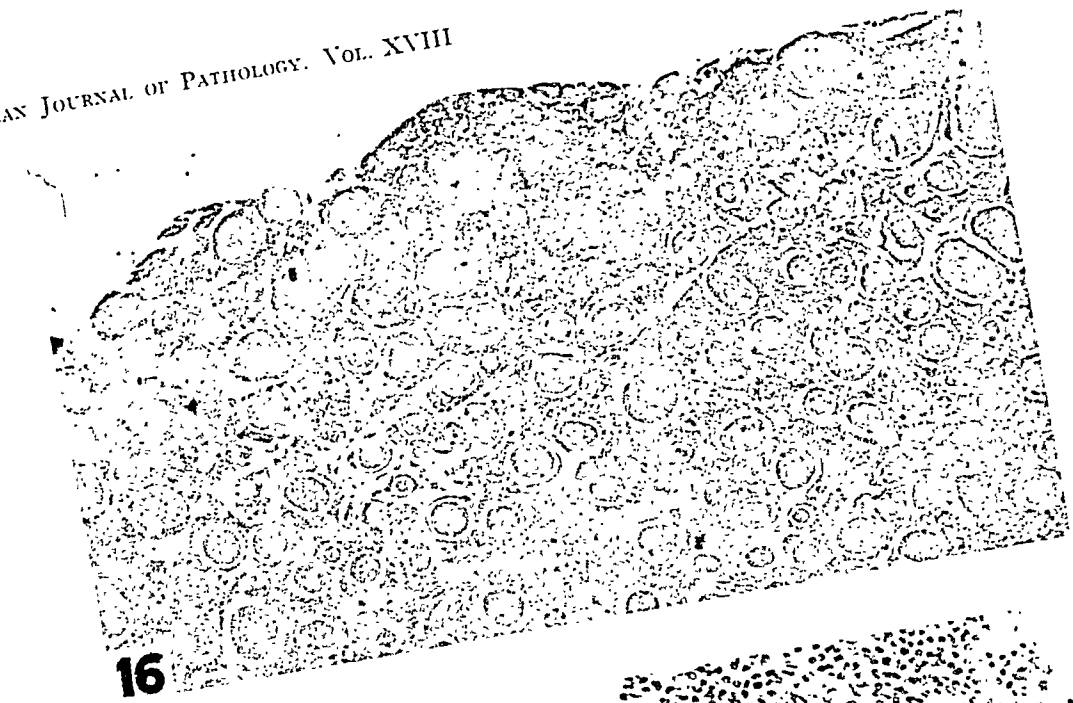
Plate 66

Fig. 13. (Lymph node). Hodgkin's lymphoma, scirrhous type. Broad strands of fibrous tissue infiltrate among the tumor cells, producing islet arrangement. × 150.

Fig. 14. (Lymph node). Hodgkin's sarcoma. The lesion consists almost wholly of Sternberg-Reed cells with a background of fibrous tissue. Relatively few mature cells are present. × 200.

Fig. 15. (Lymph node). Hodgkin's sarcoma. Giant cells with bizarre, irregular and multiple nuclei are apparent. The only other recognizable elements are fibroblasts. × 1000.

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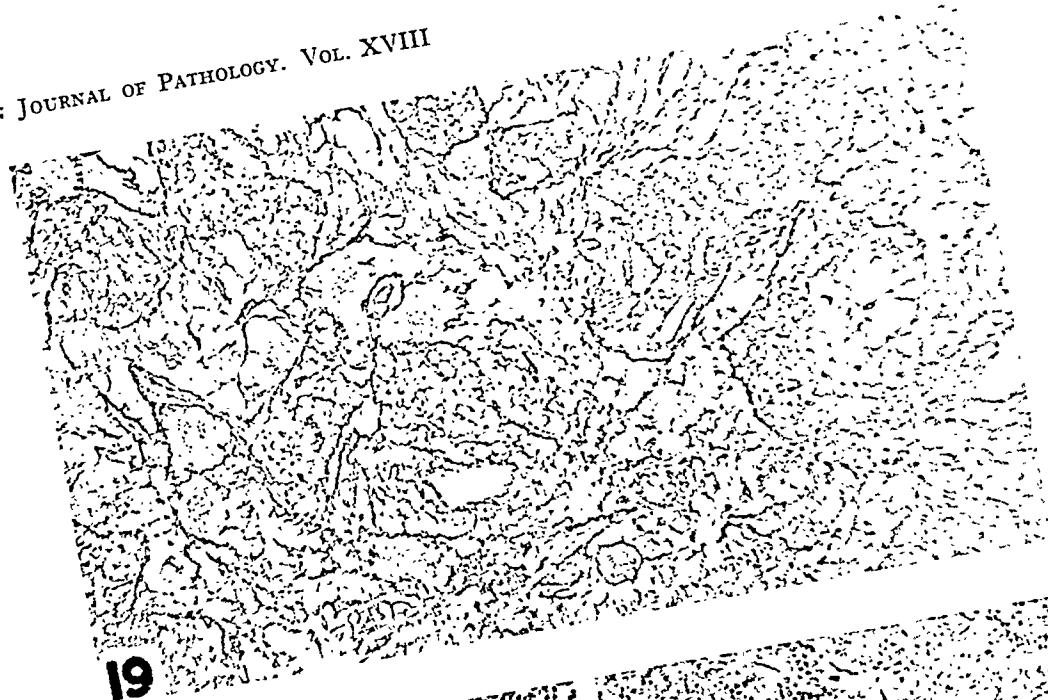
PLATE 67

FIG. 16. (Lymph node). Follicular lymphoma. Normal architecture is revised by considerably increased numbers of follicles which vary considerably in size. Clear spaces surrounding each one represent the "cracking off" phenomenon described in the text. × 50.

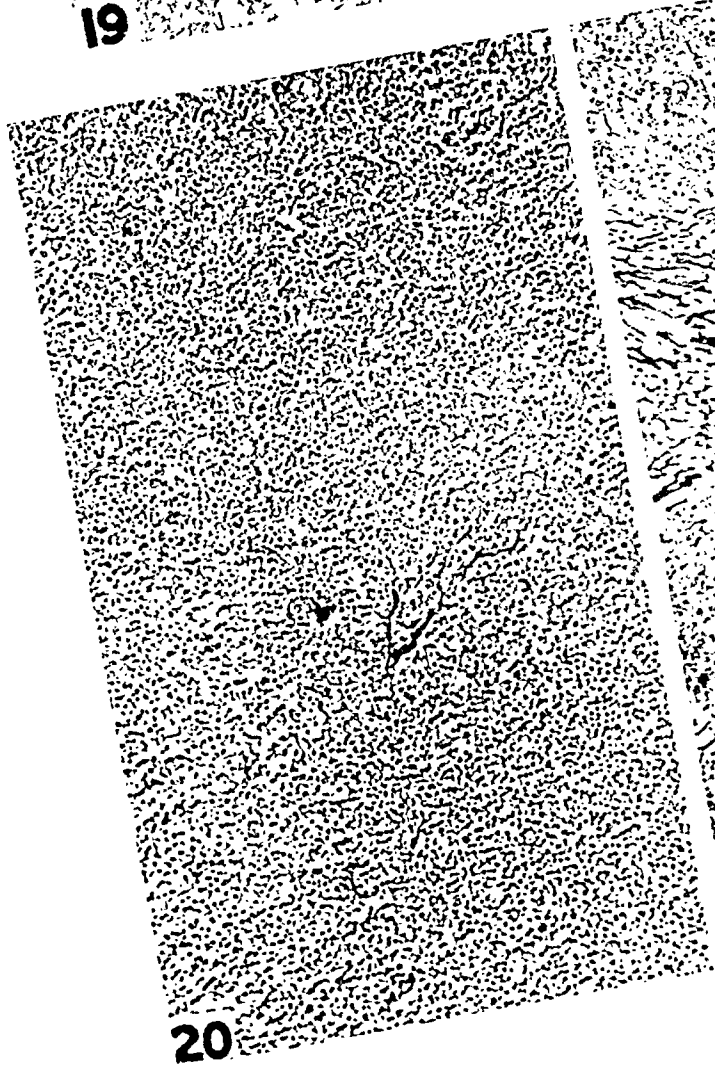
FIG. 17. (Spleen). Follicular lymphoma. Follicles are enormous and extremely irregular in configuration. Fusion of adjacent follicles may be seen. × 50.

FIG. 18. (Lymph node). Follicular lymphoma. Similarity of cells (small lymphocytes) in the pulp and follicles may be seen. The "cracking off" phenomenon is evident. × 300.

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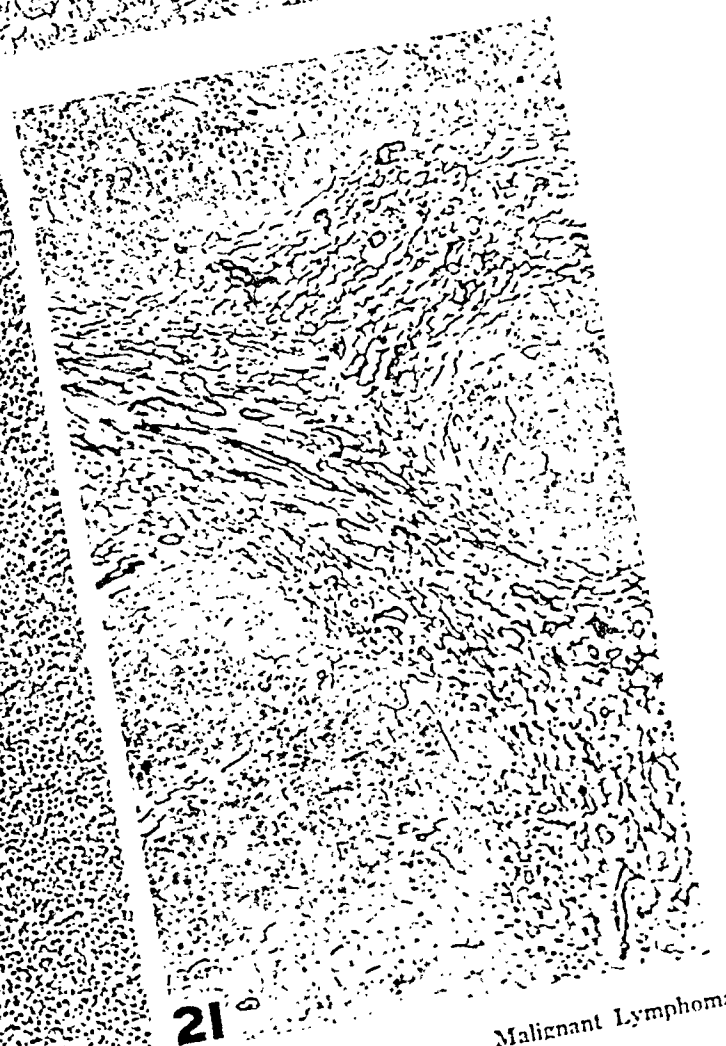


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PLATE 68

FIG. 19. Normal lymph node (Perdrau silver stain). The fine fibrillar framework is well shown and there is condensation at the edge of the sinuses. Although present in both follicles and sinuses, reticulum is scanty in these regions. × 200.

FIG. 20. Lymphocytic lymphoma (Perdrau silver stain). The normal meshwork has been replaced and only a few, fine argentophilic fibrils are seen. × 200.

FIG. 21. Follicular lymphoma (Perdrau silver stain). Stromal reticulum is obviously compressed and interfibrillar spaces narrowed and elongated. Sinuses are not seen. Reticulum content of follicles is remarkably scanty. × 200.

THE FIBROUS CONNECTIVE TISSUE OF THE ARTIFICIALLY INDUCED MATERNAL PLACENTA IN THE RAT WITH PARTICULAR REFERENCE TO THE RELATIONSHIP BETWEEN RETICULUM AND COLLAGEN *

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(From the Departments of Anatomy and Pathology, Albany Medical College, Union University, Albany, N. Y.)

Previously we [1,2] have described changes associated with advancing age in the connective tissue of the uterus of the rat. It was ascertained that beginning early in life (approximately 10 days) there was a transformation of reticulum into collagen, which was largely completed in the endometrium by 90 days of age. However, throughout life, there was a progressive deposition of additional collagenous connective tissue. Thus, for rats of different ages, it was found that the uterine connective tissue, particularly in the endometrium, assumed fairly characteristic patterns.

In this study we were impressed by one constant finding: stromal cells in the endometrium were much more abundant in those areas where reticulum was found than in those in which the fibers were collagenous. Since it is generally agreed that connective tissue cells play a dominant rôle in the origin of fibers and since it is quite likely that these cells exert a definite effect on the fibers throughout life, it was considered of some interest to ascertain what would happen to the endometrial connective tissue fibers if the structure and activity of the stromal cells were markedly altered. This was easily accomplished in the uterus of the rat by the production of maternal placentae (deciduomata †) by means of irritation. Under such conditions it is well known that the stromal cells undergo marked proliferation and hypertrophy to form decidual cells. Our purpose, therefore, was to study the fibrillar material found in deciduomata and to correlate the changes which occur in the endometrial connective tissue fibers with those which occur in the stromal cells.

Since the findings reported in this paper have a bearing on the general problem of the relationship between reticulum and collagen, several facts pertinent to this subject should be considered. In 1891, Mall [3] described fine connective tissue fibrils in many organs which branched

* Aided by grants from the Josiah Macy, Jr., Foundation and the International Cancer Research Foundation.

Received for publication, July 1, 1941.

† Although the term deciduoma implies a uterine neoplasm made up of decidual cells, it is also used to denote the mass of decidual cells (maternal placenta) that can be made to appear in the uteri of laboratory animals by artificially stimulating the endometrium when the uterus is under the influence of the hormone of the corpus luteum. The term is used in this sense in this paper.

On the fifth day of pseudopregnancy a uterine biopsy was obtained from the right horn of the uterus (deciduomata were usually found later at the cut surfaces). Deciduomata were stimulated in the left horn at two sites by passing threads through the uterus and at one site by making a longitudinal slit, approximately 5 mm. in length. Animals were killed at 3, 5, 8 and 10 days after uterine irritation (8th, 10th, 13th and 15th days of pseudopregnancy, respectively). A total of 102 deciduomata were found and studied. Those induced by slitting were generally the largest while those at the site of biopsies were the smallest; those induced by threading were intermediate. The pattern of the decidual changes occurring in the latter most nearly resembled those found in deciduomata which were induced without mechanical injury.[15] Therefore, our description is based largely on this type.

At autopsy, approximately half of the deciduomata were fixed in a 10 per cent solution of neutral formaldehyde, the remainder in Bouin's fluid. Interrupted serial sections were made from each deciduoma. Sections from those fixed in formaldehyde were so arranged that alternate ribbons of five sections were placed on separate sets of slides. One set of these was stained by Mallory's triple stain, while the second was stained by Gömöri's [16] modification of the Bielschowsky-Maresch silver impregnation method for the differentiation of reticulum and collagen. By the method of mounting, consecutive sections, one stained by the silver method and the other by Mallory's stain, could be compared. The Bouin-fixed tissue was stained only by Mallory's method. Normal uterine tissue obtained at biopsy and autopsy was fixed in both fixatives and stained by both methods.

The Gömöri [16] technic stains the reticulum black and collagen rose-red. Fibers transitional between reticulum and collagen stain purplish or reddish black. This differential staining reaction is used as the sole basis for the classification of fibrillar material. The term "argyrophilic" will be used interchangeably with "reticular." Nuclei usually stain reddish brown but in some instances are grayish. Cytoplasm is usually reddish brown but sometimes dark gray or almost black. The staining reactions of Mallory's stain are well known.

In order to study the changes in the endometrial connective tissue which occurred at the sites of deciduomata, it was considered necessary to know fairly exactly the condition of the endometrium at the time of irritation. All animals were within 2 or 3 weeks of 1 year of age at the time of autopsy. From our previous studies, the condition of the endometrium of rats of this age was well known. This control material was supplemented by the uterine biopsies, obtained at the time of irritation, which made it possible to study, in the case of each rat, a sample of the very endometrium in which the deciduomata subsequently developed.

and formed anastomosing networks. He called these fibrils reticulum and differentiated them from collagen by means of chemical and digestive experiments.[4] He considered them identical to the fine fibrils (Gitterfasern) of the liver which had been described by Kupffer,[5] in 1876, and which Oppel,[6] in 1890, had reported could be impregnated by his silver chromate method. In 1905, Maresch [7] modified the Bielschowsky silver technic for use on non-nervous tissue and in the next few years it was applied to many organs by numerous investigators.[8,9] It was found that following this procedure the fine connective tissue fibrils stained black whereas the characteristic collagenous bundles stained old rose or reddish (yellow or golden if the gold chloride bath was omitted). It has been generally assumed that the fibers which Mall has designated as reticulum are identical with the argyrophilic fibers. Furthermore, this staining reaction has come to be generally accepted as a method of differentiating reticular from collagenous fibers.

There has been much discussion concerning the ultimate relationship between the two morphologic types of connective tissue and at the present time there are two schools of thought concerning this problem. Mallory and Parker [10] and others have presented evidence indicating that the only difference between these fibers is physical. According to this view, the fine fibrils are easily impregnated and therefore are argyrophilic, while the larger bundles are not affected because of their thickness. On the other hand, Foot [11] supports the original views of Mall and thinks that the difference in staining reaction is due to chemical differences in the fibers. Regardless of the exact nature of this difference, it seems generally accepted that reticulum is a precollagenous type of tissue which may be transformed into collagen,[12-14] the fibers in the transitional state staining blackish red or purple. As far as we are aware, no work has been published which would indicate that this transformation is reversible.

Our observations indicate that during the development of deciduomata in rats, a certain amount of the endometrial collagen is transformed into reticulum. Although it was found that the width of a fiber or fibril was of importance in determining its staining reaction, there was also evidence to indicate that the location of the fiber and possibly the structure and metabolic activity of the surrounding cellular elements might play a definite rôle in determining whether or not a fiber will impregnate with silver.

Materials and Methods

Twenty-three virgin female rats were used. Pseudopregnancy was induced by mating with a vasectomized male. Vaginal smears were made daily prior to the mating and throughout the experimental period.

detailed descriptions will be given of deciduomata in rats killed 3 days following uterine irritation in which the growths were still in a developmental state, and in rats killed 8 days or more after uterine irritation in which the deciduomata, including the fibrillar material, were degenerating.

Deciduomata from Rats 5 Days After Uterine Irritation (Tenth Day of Pseudopregnancy)

The 43 deciduomata (Fig. 39) studied were composed of two general types of decidual cells: small cells (Fig. 25) generally located mesometrially and large cells (Fig. 26) most often situated at the antimesometrial pole. Transitional types were also found (Fig. 1); often cells of the same general type differed in size and appearance. Near the center of the decidual mass there was usually a small cavity or cleft containing blood (Fig. 39). This space, sometimes lined with low epithelium, represented a portion of the lumen which had been pinched off from the remainder of the cavity by the rapid growth of the decidual cells.

At the mesometrial pole the small decidual cells generally extended to the inner border of the circular smooth muscle (Fig. 39), although in some of the smaller growths a thin layer of endometrium next to the muscle layer persisted. Antimesometrially, a thicker layer of normal endometrium was usually found (Fig. 39) and this increased in thickness laterally from the center of the deciduoma toward the normal uterus on each side. The amount of the persisting endometrium at the two poles was apparently determined by the size of the decidual mass and the amount of endometrium primarily involved.

The structure of the small decidual cells was best observed in the tissue fixed in Bouin's fluid and stained by Mallory's method. Those well within the decidual mass usually differed somewhat in appearance from those near the periphery. The former were generally stellate in shape with numerous fine processes, many of which were in contact with those of neighboring cells (Fig. 25); the exact structural relationship between the processes of adjoining cells was not ascertained. Relatively wide intercellular spaces were found between the cells (Fig. 25) and their cytoplasm usually stained reddish brown and was generally, but not always; scanty. The cytoplasm was finely reticulated and often fine red granules were seen. A distinct cell membrane could not be observed. Most cells contained one nucleus, but in some two were seen; one or more prominent nucleoli were observed.

In most instances it could be demonstrated that the cells, and apparently their processes, were closely surrounded by a thin layer of blue intercellular material. In these preparations it could not be defi-

Observations

The normal endometrial connective tissue of rats of this age was found to be entirely collagenous (Figs. 21 and 23), with the exception of a reticular basement membrane, a narrow zone containing a meshwork of reticulum (subepithelial reticular zone) immediately underlying and continuous with the basement membrane (Fig. 21), and a few thin, reticular fibrils at the bases of gland cells and around blood vessels (Fig. 23). Thus, the collagenous zone of the endometrium extended from the inner border of the circular smooth muscle almost to the lumen and comprised about 80 to 90 per cent of its thickness. The collagen was arranged either as a dense meshwork of fibers, 2 to 4 μ in width, or as thicker fibers, up to 10 μ, concentrically arranged around the glands (Figs. 23 and 24) or lying in fairly regular rows more or less parallel to the circular smooth muscle (Fig. 23). The fibers were thickest and most densely arranged deep in the endometrium (Fig. 23). As the lumen was approached, thinner fibers arranged in a meshwork were encountered and at the lower limits of the subepithelial zone they blended almost imperceptibly with the reticular fibrils (Fig. 21).

Stromal cells of the endometrium were definitely most abundant in the subepithelial zone of reticulum (Fig. 22). In the collagenous portion they were less numerous and in the deeper parts, where the collagenous fibers were wider and more densely arranged, they were so few as to be inconspicuous. The cells lying between the thicker fibers were often compressed and spindle-shaped.

Structural details of deciduomata in rats have been described by Selye and McKeown [17] and Krehbeil.[15] They have shown these growths to be composed of two general types of decidual cells: small glycogen-containing cells located at the mesometrial pole, and larger cells which contain lipoid material located at the antimesometrial pole. We have found both of these types with intermediate variants between them. In regressing deciduomata, Selye and McKeown also described large granular cells in the mesometrium, forming a structure they called the "metrial gland," a finding we have confirmed. Although the present paper is concerned chiefly with fibrous tissue, we have studied the more general histologic details of deciduomata, including growth and degeneration. Our general observations largely confirm and in some instances supplement those of the writers cited.

It is known that deciduomata reach the peak of their development about 5 days after uterine irritation (tenth day of pseudopregnancy). These we are describing in detail in order to establish the normal pattern of the fibrillar material in the fully developed deciduoma. Less

the fibrillar elements of deciduomata, namely, a marked tendency for the fibers to branch or to become separated into finer elements. This occurred among reticular, transitional and collagenous elements. Often the finer fibrils, partially separated from the thicker fibers, were more argyrophilic than the parent fiber (Figs. 7, 8 and 11). Quite frequently, the lateral borders of the fibers were stained black while the central portion was transitional or collagenous (Figs. 5 and 13). The significance of branching and of the staining reaction will be considered later.

The relationship between the fibrillar material and the small decidual cells can best be understood by studying Figures 27 to 32. Although there was some tendency toward a characteristic arrangement of fibrillar material throughout the entire area occupied by the small decidual cells, there was, nevertheless, a definite variation in the relationship of these elements to the decidual cells. Because of this variation, we will describe several of the most common structural patterns found.

The most typical arrangement is shown in Figure 28. It is seen that in such areas the fine reticular fibrils lined the large and prominent intercellular spaces and were also closely applied to the decidual cells and their processes. The fine argyrophilic "granules" were considered to be fibrils cut transversely. Study of such areas as these strongly indicates that the intercellular material, which in the Mallory preparations was applied to the small decidual cells, was fibrillar. The thicker fibers are well shown in this section. In contrast to the thin fibrils they did not seem to bear any constant relation to the cells. As we have stated before, these thicker fibers were sometimes reticular, but most often they were either transitional or collagenous. This is the type of fiber already referred to which did not photograph well in the Mallory preparations.

Another structural pattern, found almost as frequently as that just described, is shown in Figure 29. The cell processes were fine and apparently anastomosed with those of adjacent cells. The intercellular spaces were small and the number of reticular fibrils found among the cells was much less than in Figure 28. Thicker fibers were also present; they were unusually abundant in this particular section.

An arrangement of the fibrillar elements and decidual cells often found adjoining the remains of the uterine lumen is shown in Figure 27. Here a very regular reticular meshwork was formed about single decidual cells. Intercellular spaces or thicker fibers were most often not present. This type of arrangement was found infrequently in this group but was quite prevalent in the 3-day group.

In Figure 30 is shown the type of structure often found near the

nitely determined whether or not this fine intercellular material was actually fibrillar. Thin strands of this substance extended out into the intercellular spaces and formed a meshwork. The intercellular spaces appeared irregularly rounded and were bounded both by cell processes and strands of intercellular material (Fig. 25). We feel that they are the sites of accumulation of fluid lying in the intercellular material. A few definite connective tissue fibers were seen; they were relatively wide, measuring up to 3 μ, and usually wavy. As will be seen later, they were actually segments of longer fibers. They stained more deeply than the finer intercellular material, but had no demonstrable spatial relationship to the cells. Since in the Mallory preparations they did not come into good focus at the same point as the decidual cells, it was difficult to demonstrate them in photomicrographs of these sections.

Near the periphery of the deciduoma, the small decidual cells were generally more compactly arranged, the cell processes being less abundant, but shorter and thicker and often appearing continuous with those of adjoining cells. The intercellular spaces were less numerous, but generally large and irregularly rounded. Fine strands of intercellular material, similar to that described above, closely surrounded the cells and the intercellular spaces.

In the silver (Gömöri) preparations, a much clearer concept of the nature of the intercellular material among the small decidual cells could be gained (Figs. 27 to 32). The fibrillar elements, which in most instances were quite wavy, ranged from very fine fibrils to fibers up to 3 μ in thickness (Figs. 2 to 14 and 27 to 32). All but the very finest often showed a cut surface at each end which was slightly expanded (Figs. 4, 7 and 8). Generally speaking, fibrillar material was more abundant at the periphery of the decidual mass where it blended with that of the adjoining endometrium (Figs. 35 and 36).

The fine fibrils were almost invariably argyrophilic and were therefore considered to be reticulum (Figs. 2, 3, 11 and 31). In some instances the thick fibers (2 or 3 μ in thickness) were also argyrophilic (Figs. 9, 10, 14 and 28); in others they were either collagenous (Fig. 13) or transitional between reticulum and collagen (Figs. 4, 7, 8 and 29). Thin fibers (approximately 1 μ in diameter) were generally reticular (Fig. 10) or transitional (Figs. 5 and 11). These observations are of considerable interest when it is remembered that in the normal endometrium only the finest fibrils (only a fraction of 1 μ thick as determined by methods not absolutely accurate) were argyrophilic, while thicker elements were invariably collagenous.

Study of the various figures showing silver preparations, and particularly Figures 2 to 14, will reveal a definite structural characteristic of

between the large and small decidual cells. In this transitional area the decidual cells became smaller and less closely packed, and intercellular spaces identical with those among the small decidual cells appeared between them (Fig. 1). Cell processes were generally in contact with those of adjoining cells and often there appeared to be cytoplasmic continuity as well. Abundant light blue intercellular material was in contact with the cells and formed a fine weblike mesh between them and the wide intercellular spaces (Fig. 1). In some instances, irregular masses of fine "granular-appearing" intercellular material, possibly coagulated intercellular fluid, were found between the cells. The intercellular material as it appeared in the Mallory preparations could not definitely be described as fibrillar. In addition to the weblike intercellular substance, a few thick fibers were found. They stained more darkly and were definitely fibrillar. In the silver sections, the intercellular material between these transitional cells had a different appearance. Fine fibrils surrounded the cells and reproduced to a certain degree the fine meshwork observed in the Mallory preparations. No argyrophilic material resembling the "granular-appearing" substance mentioned above was noticed. The thick fibers were prominent.

The structure at the junction of the decidual growth and the normal endometrium varied, two main types being found (Figs. 20, 35 and 36). In the first, only a narrow transitional zone appeared between the decidual growth and the decidual endometrium and the separation between the two types of tissue was fairly sharp and distinct. In this type there was a tendency for the more peripheral decidual cells to be compressed. Outside of these, there was often a narrow zone which blended with the adjoining endometrium in which the stromal cells were slightly hypertrophied.

The second and more common type of junction was much less clear-cut and extended throughout a relatively wide transitional zone (Figs. 20, 35 and 36). In this zone the cells showed moderate hypertrophy, which was greatest immediately adjoining the decidual growth and became progressively less in the adjacent endometrium. The fibrillar material presented comparable changes. The broad and dense collagenous fibers of the adjoining endometrium lost their regular pattern and many became split up into finer elements to form an irregular meshwork (Figs. 35 and 36) about the moderately altered cells. The finer elements were either reticular or transitional in nature; the coarser were collagenous. In many instances broad collagenous fibers extended into the peripheral portions of the deciduomata where they split into finer units (Fig. 36).

The observations already recorded furnish considerable evidence

periphery of a deciduoma. The cells were somewhat irregularly arranged and often appeared fused. The intercellular spaces were relatively large and in many places fine reticular fibrils were in contact with the cells and formed a lining for the irregular spaces. As usual the thicker fibers were irregularly arranged in relation to the decidual cells. Structural conditions similar to those shown in Figures 31 and 32 were less often observed near the peripheral portion of decidual growths. The fibrillar elements were variable in size and followed no definite pattern in their relationship to the decidual cells. The branching and the variable form of the fibers are well shown in these two figures.

These observations indicate that there was a tendency for fine reticular fibrils to lie in close contact with the small decidual cells and at the same time to form a lining for the intercellular spaces. However, this was not a constant finding; in some areas there did not seem to be sufficient reticulum present to make such an arrangement possible (Fig. 29), and in other areas there was no constant structural relationship between the cells and reticulum fibrils although the latter were present in large numbers (Fig. 31). In no instance were the thicker fibrillar elements found to bear a constant relationship to the small decidual cells.

The large decidual cells stained by the Mallory method are pictured in Figure 26. They were usually polygonal in shape and closely packed. In general, in those cells which were in contact with each other, cell membranes were visible; sometimes they were not. On the other hand, cell membranes could not be demonstrated in cells which occurred singly, a finding for which we have no explanation. The cytoplasm usually stained reddish brown, appeared finely reticulated and often contained small red granules. The cells were often binucleate with prominent nuclear membranes and large nucleoli. In the silver preparations (Fig. 33) it was found that fibrillar material among these cells was generally much less abundant than among the small decidual cells, although in some instances it was present in considerable amounts among those cells located near the periphery of the decidual mass (Fig. 34). The fibrils were most often thin and reticular although thicker fibers, either transitional or collagenous, were observed. The fibers passed between or over the decidual cells and apparently formed no definite pattern.

Decidual cells which were transitional between the small and large types were found near the junction of the mesometrial and antimesometrial regions (Fig. 39; area marked by arrow). In the Mallory preparations they were of intermediate size and formed a graded series

most of the smaller deciduomata the small-cell area, located mesometrially, involved more of the endometrium at proximal and distal extremities of the deciduoma than did the large-cell area situated on the antimesometrial side.

With two exceptions, both large and small decidual cells were present in the deciduomata of this group and, although there were some variations, the small cell-areas seemed better developed. In two very weak reactions, only small cells were present, both on the mesometrial side. These two do not necessarily indicate that this area is the normal site of the primary focus of decidual formation. In fact, Krehbeil, [15] who has studied deciduomata induced without mechanical injury, stated that the primary focus is on the antimesometrial side. Regardless of its mesometrial or antimesometrial situation, we believe, with Krehbeil, that the first decidual cells appear immediately under the lumen in the subepithelial reticular zone (Fig. 41).

The decidual cells, both small and large, in deciduomata of this group, were generally not as large as the corresponding types in the 5-day group. The condition of the fibrillar material, however, was similar, although the regular meshwork about the cells, as shown in Figure 27, was much more frequently found than in the 5-day group, and most frequently in areas fairly close to the lumen or its remnant. It should be remembered that in the normal uterus a regular reticular meshwork is found immediately underlying the lining epithelium (Fig. 21) and below this there is usually a meshwork of fairly thin collagenous fibers. Evidently the relatively more regular arrangement of the fibrillar elements of this area of the normal endometrium results in a more regular reticular meshwork about decidual cells that form here.

Changes in the endometrium immediately adjacent to the transitional zone were often striking. One type of change is shown in Figure 20. The light areas in the endometrium of this section represent sites at which the broad collagenous fibers (Fig. 23) commonly found in this region have disappeared and have been replaced by a sheet of finely fibrillar collagen which stained light blue in the Mallory preparations (Fig. 38) and a light shade of old rose by the Gömöri method (Fig. 37). Although the individual fibrils encountered in these areas were as thin as the finest reticular fibrils found in the deciduoma, they were collagenous rather than reticular. The significance of this finding will be discussed later. The stromal cells were moderately hypertrophied. In Figure 38, it is noted that remnants of the broad collagenous fibers persisted and that they stained more deeply than the finer fibrillated material surrounding them. In such areas of endometrium adjoining the decidual mass, the broad collagenous fibers around the glands

concerning the origin of the fibrillar material of deciduomata. The well developed deciduoma involves practically all of the endometrium, both mesometrial and antimesometrial, at the site of its formation. In rats 1 year of age, this endometrium is almost completely collagenous. At the periphery of the growth in the transitional zone there was evidence that the broad collagenous fibers were being divided into finer fibers and even fibrils, while in the interior of the decidual mass the fibers present appeared to be splitting into their component fibrils. We have therefore come to the conclusion that, during the development and growth of the deciduoma, the normal pattern of the endometrial connective tissue fibers, practically all of which are collagenous, is completely altered, apparently by a process in which the fibers are split into much finer elements which are generally transitional or reticular in nature. The fibrillar material is then reorganized into the pattern found in the deciduoma. The coarse fibers present are apparently those which have not been reduced to smaller units.

Deciduomata from Rats 3 Days after Uterine Irritation (Eighth Day of Pseudopregnancy)

The 31 deciduomata studied in this group were variable in size; some could scarcely be seen while others were as large as those in the 5-day group. Histologically, all of these, particularly the smaller, were less sharply delineated from the surrounding endometrium, the transitional zones being relatively wide in comparison with the size of the decidual masses themselves. Furthermore, structural changes in the endometrium adjoining the zone of transition were found, probably because this endometrium was destined later to be incorporated into the decidual mass.

As the deciduoma was approached from the normal uterus on either side, the first change usually noted was some increase in the width of the subepithelial zone of reticulum, usually most pronounced at the mesometrial pole. The most peripheral decidual reaction first appeared immediately under the lining epithelium in this region (Fig. 41). Progressing from this point inward, the decidual reaction appeared to radiate laterally and antimesometrially as well as mesometrially, and rapidly became larger (Figs. 15 and 18). As this occurred, more and more of the endometrium became involved. The collagenous connective tissue fibers became split into finer elements and became increasingly argyrophilic, although fairly wide fibers often extended quite a distance into the growth (Fig. 36). A mass of decidua was pushed into the lumen from the mesometrial side (Fig. 15); this presumably was the mechanism by means of which the uterine lumen was occluded. In

area. In those areas in which the degenerative process was not yet marked, the fibrillar material was not greatly different from that found in the 5-day group. However, since the intercellular spaces had largely disappeared, there was a greater irregularity in the relation of the fine fibrils to the cells.

In areas in which the process of degeneration had progressed to the stage of cellular disintegration (Fig. 17; immediately surrounding the lumen) the fibrillar substance showed pronounced and fairly constant changes. The thicker fibers lost their regular, compact appearance. Many fine fibrils projected from their lateral borders, giving them a shaggy appearance (Figs. 12 and 19; fibrils indicated by arrows). It appeared that the mechanism by which many fibrils are bound together to form a fiber had failed and that the fibers were breaking down into their component fibrils. Although these fibrils were extremely thin, they were usually collagenous (Figs. 12 and 19); however, in many instances their tips were argyrophilic (Fig. 12).

In other areas great numbers of fine fibrils formed large, irregular, tangled masses lying among, but not definitely associated with, the degenerate decidual cells. These fine fibrils usually stained rose-red; less frequently they were weakly argyrophilic. Fibrils of this size in a healthy deciduoma would almost invariably be reticular in nature. Therefore, in the degenerating decidual tissue, there was a distinct loss in the capacity of the fibrillar material to be impregnated by silver salts, although fine fibrils retained this capacity to a certain extent. Our observations would indicate that the degenerate fibrillar material was lost along with the necrotic cellular mass.

Beginning at about the same time as the degenerative changes described above, an enlargement occurred in the mesometrium (Fig. 40). The predominant cells in this swollen area were large, contained many red granules which were irregular in size and distribution and had irregular processes. These cells were arranged about the blood vessels forming a series of lobules (Fig. 40). This enlargement, called the "metrial gland" by Selye and McKeown,[17] has been described in detail by them. Therefore, our description will be limited chiefly to the fibrillar material.

In the normal mesometrial region (Fig. 16; taken from a 3-day deciduoma) blood vessels were abundant. They were surrounded by narrow collagenous fibers, although immediately beneath the endothelium reticular fibrils were present. Lying between the blood vessels were fine, closely spaced collagenous fibrils arranged in a fibrillated sheetlike mass. Stromal cells were situated between the perivascular collagenous fibers and in the finely fibrillated collagen between the vessels. In the 5-day deciduomata, these stromal cells, as well as those

showed a marked tendency to persist (Fig. 20) while those more remotely situated from the glands were replaced by the finely fibrillated material.

In the zone of transition seen in Figure 20, the moderately hypertrophied stromal cells blended so gradually with those of the decidua that it was impossible to ascertain exactly where endometrium ended and decidua began. Here most of the broad collagenous fibers ordinarily found in the endometrium were replaced by generally thinner and more irregularly arranged elements.

Deciduomata from Rats 8 to 11 Days after Uterine Irritation (13th to 16th Days of Pseudopregnancy)

The 28 deciduomata from these rats were considered as a single group because of common structural characteristics; *i.e.*, degenerative changes and formation of the "metrial gland" (Fig. 40).

Degenerative changes in the decidual cells were found as early as 8 days after uterine irritation and became progressively more marked in the older growths. The changes occurred first in the large decidual cells bordering the remains of the uterine lumen and involved the smaller cells secondarily. As these changes progressed, the degenerated tissue was sloughed into the remains of the uterine cavity, which became larger as more and more decidua disappeared (Figs. 17 and 40). At points at which the degenerative process was complete, an epithelial lining reappeared in the lumen (Fig. 40). In most of the deciduomata of this group, the large cells had already disappeared but varying amounts of the small-celled decidua were found in all (Fig. 40). Rats killed during this period showed a bloody vaginal discharge similar to that found when spontaneous fetal resorption was occurring.[18]

The degenerative process involved shrinkage of the cells and pyknosis of the nuclei in both the large and the small decidual cells (Fig. 17). In the small-celled areas, the intercellular spaces disappeared and the cells became more closely packed. The sinusoids immediately under the lumen on the mesometrial side were generally distended. Often their walls were broken down and there was hemorrhage into the structureless mass lying in this region (Figs. 17 and 40). The type of degenerative process described here appeared to be similar to that which occurs in the placenta during spontaneous fetal resorption in the rat [18] and it is believed that the underlying mechanism is the same in both instances.

The appearance of the fibrillar material was somewhat variable and difficult to analyze. However, the structure of the fibers and fibrils and their affinity for the stain depended, at least to a certain extent, on the degree of cellular degeneration which had occurred in that particular

during the course of development of the deciduoma. There seems to be a close parallelism between the cellular and fibrillar changes; as the stromal cells hypertrophy the fibrillar material is completely reorganized into the pattern found in the deciduoma. Apparently this is accomplished by a division of the thick, endometrial, collagenous fibers into thinner fibers and fibrils, which become either transitional or reticular in nature. The finer elements usually come to be arranged in a more or less complete meshwork about the decidual cells. This study therefore presents definite morphologic evidence that endometrial collagen is transformed into the reticular and transitional fibers found in deciduomata. This view is further supported by two negative findings. There was only a very small amount of preëxisting reticulum in the endometrium in which the deciduomata were stimulated and we could find no evidence that this accounted for the large amount of reticular material in the decidual growths. There was also no evidence of the deposition of new fibrillar material which could account for the large amount of decidual reticulum. It is already generally recognized that reticulum may be transformed into collagen and these studies furnish histologic evidence that under certain circumstances collagen may be changed into reticulum. It would seem, therefore, that the fibrous, intercellular material of the endometrium is quite labile and subject to change under the proper conditions. It will be of great interest to see if this is true in connective tissue generally.

It has already been indicated that the exact relationship of reticulum and collagen is a controversial matter. Therefore, certain of our findings, which might shed some light on the subject, should be pointed out. In deciduomata a fairly definite relationship between the thickness of a fiber and its reaction to the silver stain was found. In normal decidual tissue the fibrils and thinner fibers were argyrophilic, while the thicker elements generally took a stain characteristic of collagen or transitional material. The same holds true in the normal endometrium; the fine elements immediately under the lining epithelium stained black and were considered to be reticulum, while the thick fibers deep in the endometrium were rose red, which indicated their collagenous nature. To a certain degree, therefore, our findings would tend to support the views of Mallory and Parker.[10] However, certain other observations indicate that the thickness of a fiber is not the only factor which determines whether it will or will not be impregnated with silver. In some deciduomata it was found that coarser fibers, up to 3 μ in thickness, were sometimes argyrophilic and thinner fibers of approximately 1 μ in width were quite regularly stained black. Fibers of these thicknesses in the normal endometrium were almost invariably collagenous. Furth-

lying between the muscle fibers of the circular smooth muscle in this region, were already hypertrophied. Hypertrophy in the latter situation caused a separation and some scattering of the muscle fibers. In the present group, this condition was marked and established a more or less direct connection between the metrial gland and the decidual mass (Fig. 40). These preliminary changes apparently preceded the formation of the well developed metrial enlargement.

The fibrillar material of a portion of a single lobule of the fully developed metrial enlargement is shown in Figure 42. The perivascular connective tissue was collagenous. Arranged about the cells was an irregular meshwork of fibrils and thin fibers. Occasionally the fibrillar material about the cells was definitely reticular (Fig. 42) but in most instances these fibers stained purplish or blackish red and were considered to be transitional in nature. Separating the lobules were more densely arranged and thicker collagenous fibers. Often the lobules were irregularly formed and not well separated from each other. Our studies would indicate that the fibrillar material in the metrial enlargement represented a modification of the connective tissue fibers, practically all of which are collagenous, normally found in the mesometrial region.

The origin and significance of the granular cells of the mesometrial region have been considered by Selye and McKeown.[17] These writers reported that they have a widespread origin and may arise from several types of cells found in this region; *i.e.*, smooth muscle cells, endothelial cells and fibroblasts. Although our observations are more limited, we think that their origin is not so general. As already indicated, all stromal cells in this region, including those lying between the blood vessels and those lying between the smooth muscle fibers, showed a definite hypertrophy, and in the rats with degenerating deciduomata it was possible to find transitional forms between the decidual cells and the granular cells. We are, therefore, of the opinion that these granular cells are derived only from connective tissue cells or from their derivatives.

Discussion

Two questions of interest arise as a result of these studies. They are concerned with the nature of the changes which occur in the fibrous material of the endometrium and the factors which determine the staining reaction of these connective tissue fibers.

It is well recognized that decidual cells arise as the result of hypertrophy and proliferation of the endometrial stromal cells. This study indicates that in like manner the fibrillar elements are derived from the endometrial connective tissue fibers which are profoundly modified

collagenous reaction. Although this hypothesis is not supported by direct evidence, it does form a logical explanation for our observations.

In certain deciduomata there was a considerable tendency for some transitional or collagenous fibers to have black lateral borders (Figs. 5, 7, 11 and 13). As far as we can ascertain, such a finding has not been reported before. Since we have observed this phenomenon to a slight extent in previous studies on endometrial connective tissue and with considerable regularity in the present material, it should be mentioned. Although the significance of this phenomenon is unknown, it seems obvious that it is most easily interpreted on the basis of the hypothesis described above, namely, that the outer surfaces of the fibers are more accessible to the environmental influence and would therefore react more readily to the silver salts.

Summary and Conclusion

1. The fibrillar material in deciduomata of the rat has been studied by methods in general use for the differentiation of reticulum and collagen.

2. The fibrillar elements ranged from fine fibrils to fibers approximately 3 μ in width. The fine fibrils were almost always reticular in nature. The thick fibers were sometimes reticular, but more often they were either collagenous or transitional between reticulum and collagen. It was found that the fibrous material in deciduomata of the rat was derived from that of the normal endometrium which underwent marked changes during the development of the decidual growth. Evidence is presented to show that under certain circumstances collagen may be transformed into reticulum.

3. The relationship between reticulum and collagen is discussed and factors regulating their staining reactions are considered. It is suggested that certain localized factors might influence the staining reaction of connective tissue fibers.

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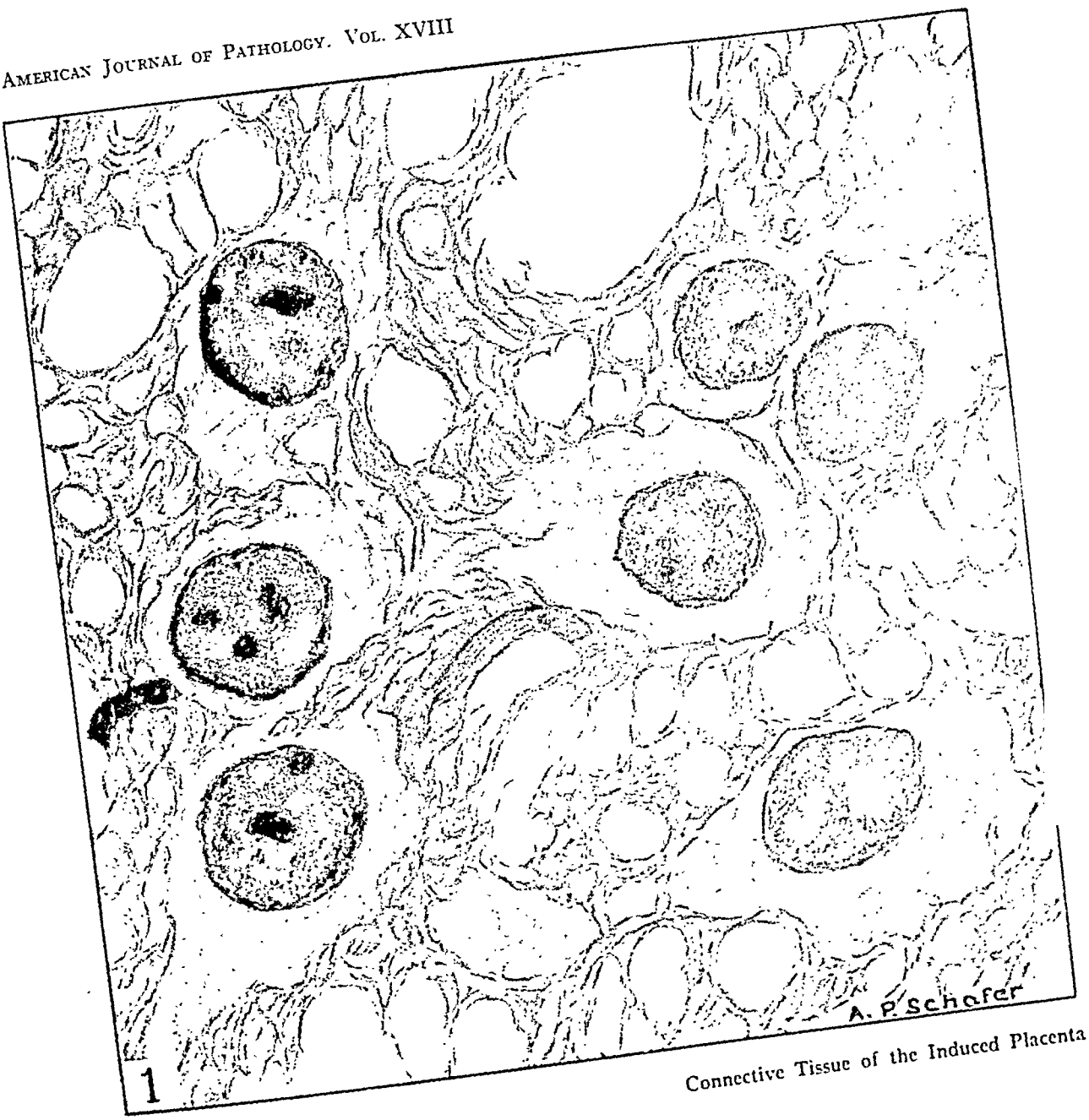
ermore, in the normal uterus, fine collagenous fibrils have repeatedly been observed in the mesometrial region and in the spaces between the two muscle layers. In the collagenous zone of the normal endometrium fine fibrils were often seen in instances in which broad bandlike fibers split up into fine fibrils (Fig. 6); both were collagenous in spite of the tremendous difference in width. Furthermore, in degenerating decidual tissue, the finest fibrils showed a marked tendency to be collagenous in nature (Fig. 12).

These observations indicate that the location of a fibril or a fiber and its relationship to the surrounding tissues may play a definite rôle in its staining reaction. Thus in healthy decidual tissue there appears to be some influence which increases the capacity of the fibrous elements to be reticular, while in degenerating decidual tissue such an influence is evidently missing. In the normal uterus fine fibrils immediately adjacent to the lumen are reticular while those which are just as fine, but are located in the mesometrial region, are collagenous. We feel, therefore, that it is legitimate to suggest that some local environmental factor may play a rôle in the staining reaction of the connective tissue fibers. Although the nature of this hypothetical environmental factor is not known, our observations would suggest that it is associated with cell activity. Thus in degenerating decidual tissue even the finest fibrils tend to lose their argyrophilia and the degree of this loss appears to be associated with the degree of regression of the decidual cells. Such fibrils in healthy decidual tissue were invariably reticular. In the normal endometrium, the cellular elements are most abundant immediately under the lining epithelium; the fibrils here are definitely reticular. Yet fibrils just as thin, located in a zone where cells are less abundant, are collagenous.

If such an environmental factor actually exists, it seems possible that it is some metabolic product of the cell which is released into the surrounding tissues and is conveyed to the fibers by the intercellular fluid which bathes both fibers and cells. To carry this concept a step further (and we emphasize that the concept is purely hypothetical), it seems possible that this substance might penetrate into the fiber to a variable degree and thus modify its permeability or its chemical nature.* On this basis, it would be entirely logical for thin fibrils to be more argyrophilic than thicker fibers. Yet, if this environmental influence is strong enough, even thick fibers might be argyrophilic or transitional, and if it is sufficiently weak, even the finest fibrils would exhibit a

*N. C. Foot (On the origin of reticulin fibrils. *Am. J. Path.*, 1927, 3, 401–412) has suggested that collagen is a colloidal or semifluid substance which permeates fibers and induces them to lose their affinity for the silver salt.

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Wolfe and Wright

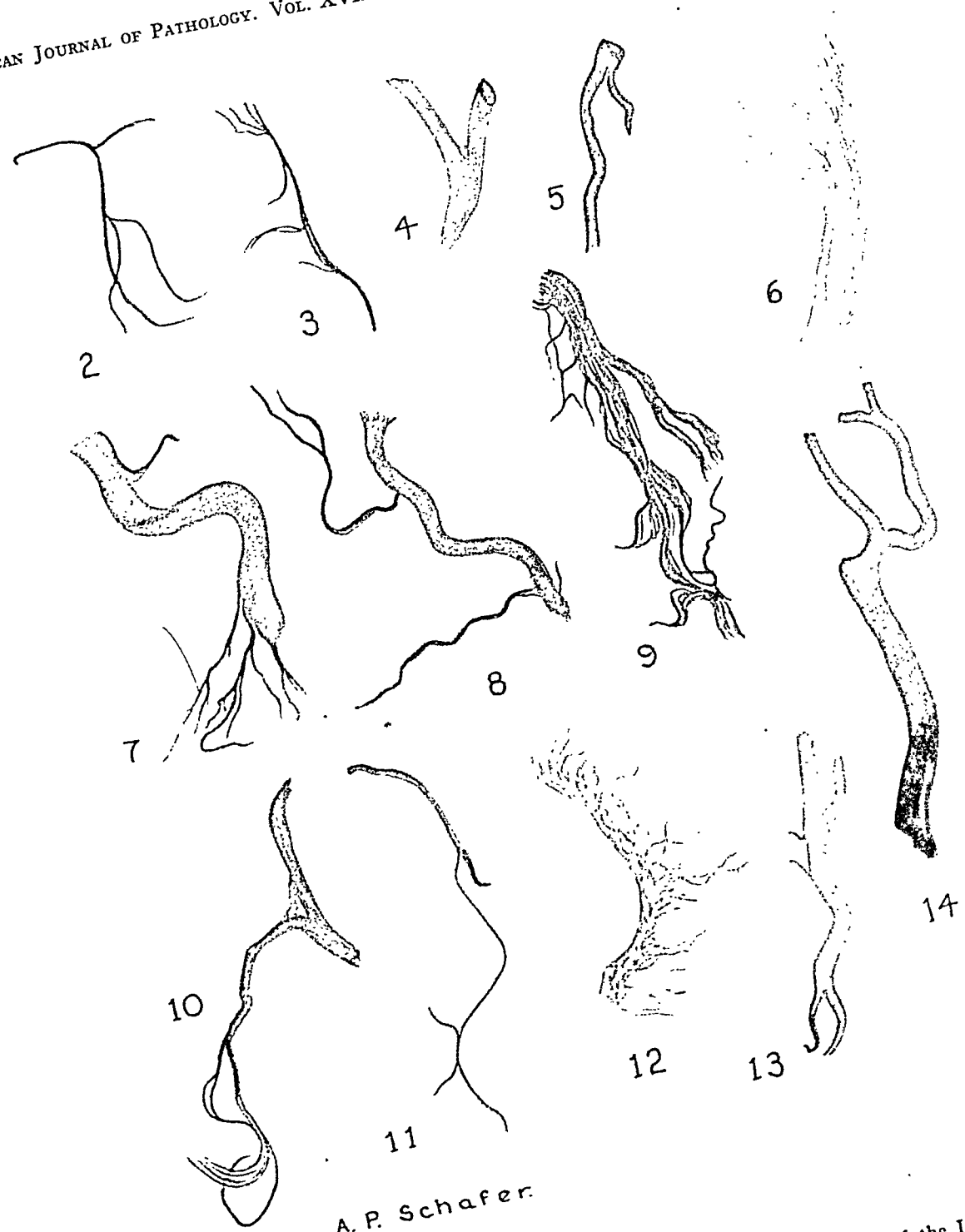
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DESCRIPTION OF PLATES

PLATE 69

FIG. 1. Pencil drawing of a section stained by the Mallory technic showing transitional decidual cells surrounded by intercellular material which in such preparations did not appear definitely fibrillar. Large intercellular spaces and portions of two thick fibers are seen. × 1950.

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PLATE 70

(All figures taken from Gömöri preparations of deciduomata unless otherwise noted.)

FIG. 2. Fine reticulum fibril splitting into finer units. × 1200.

FIG. 3. Fine reticulum fibril splitting into finer units. × 1200.

FIG. 4. Portion of a thick transitional fiber showing splitting into two thinner elements. × 1200.

FIG. 5. A thinner transitional fiber. The lateral surfaces are argyrophilic. × 1200.

FIG. 6. A collagenous fiber from normal endometrium, showing one end split into thin fibrils which are collagenous in nature. × 1200.

FIG. 7. A thick (3 μ) transitional fiber which shows argyrophilic fibrils splitting off at one end and a dark surface area. × 1200.

FIG. 8. A transitional fiber almost as black as reticulum. The thinner elements separating from the fibers are definitely argyrophilic. × 1200.

FIG. 9. A thick (3 μ) reticular fiber beginning to split into finer elements. × 1200.

FIG. 10. A moderately thick reticular fiber with finer branches. × 1200.

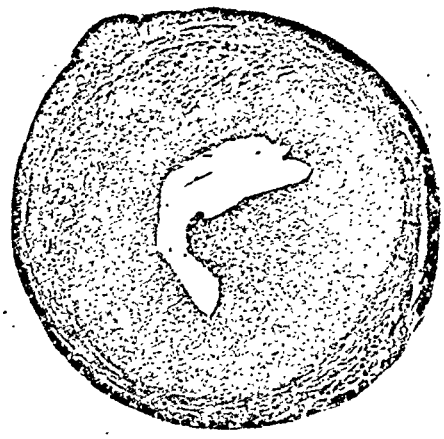
FIG. 11. A thin transitional fiber showing a fine reticular fibril coming off one side. × 1200.

FIG. 12. A collagenous fiber taken from degenerating decidual tissue (see Fig. 17). The constituent fibrils are separating. Although the fibrils are stained rose-red, their tips are weakly argyrophilic. × 1200.

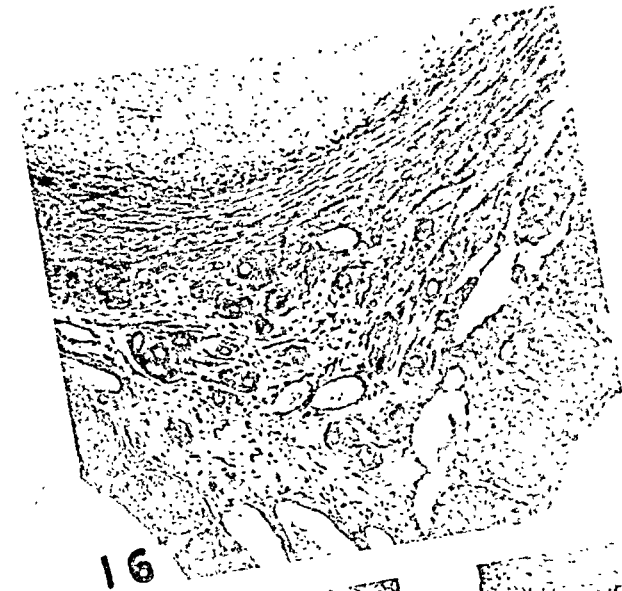
FIG. 13. A collagenous fiber (2 μ) found in normal decidual tissue. × 1200.

FIG. 14. A thick (slightly over 3 μ) reticular fiber. × 1200.

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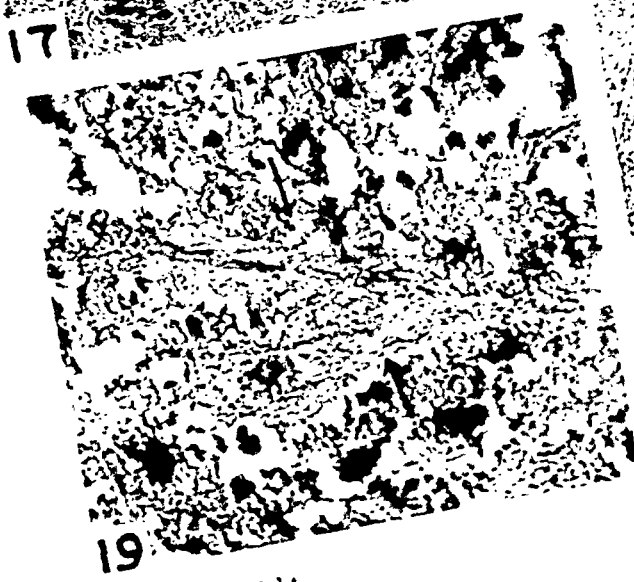
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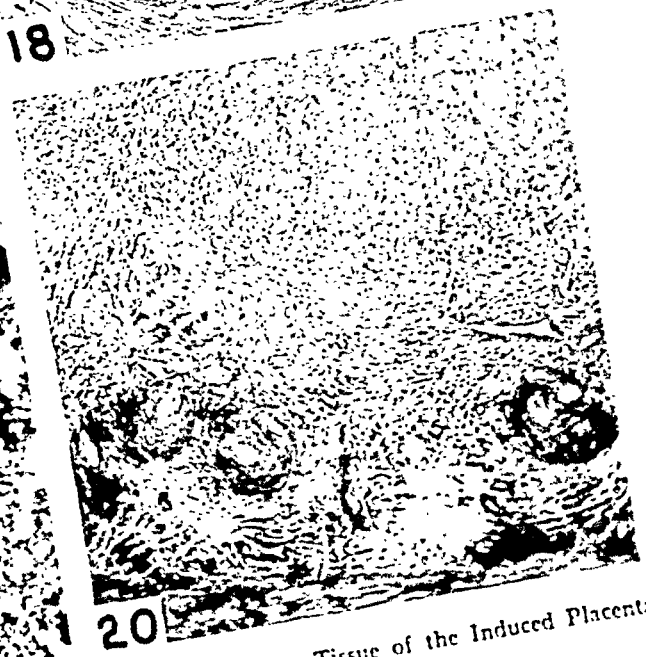
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19



20

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PLATE 71

FIG. 15. A small 3-day deciduoma. Mallory preparation. × 18.

FIG. 16. Mesometrial region of 3-day deciduoma. Mallory preparation. × 62.

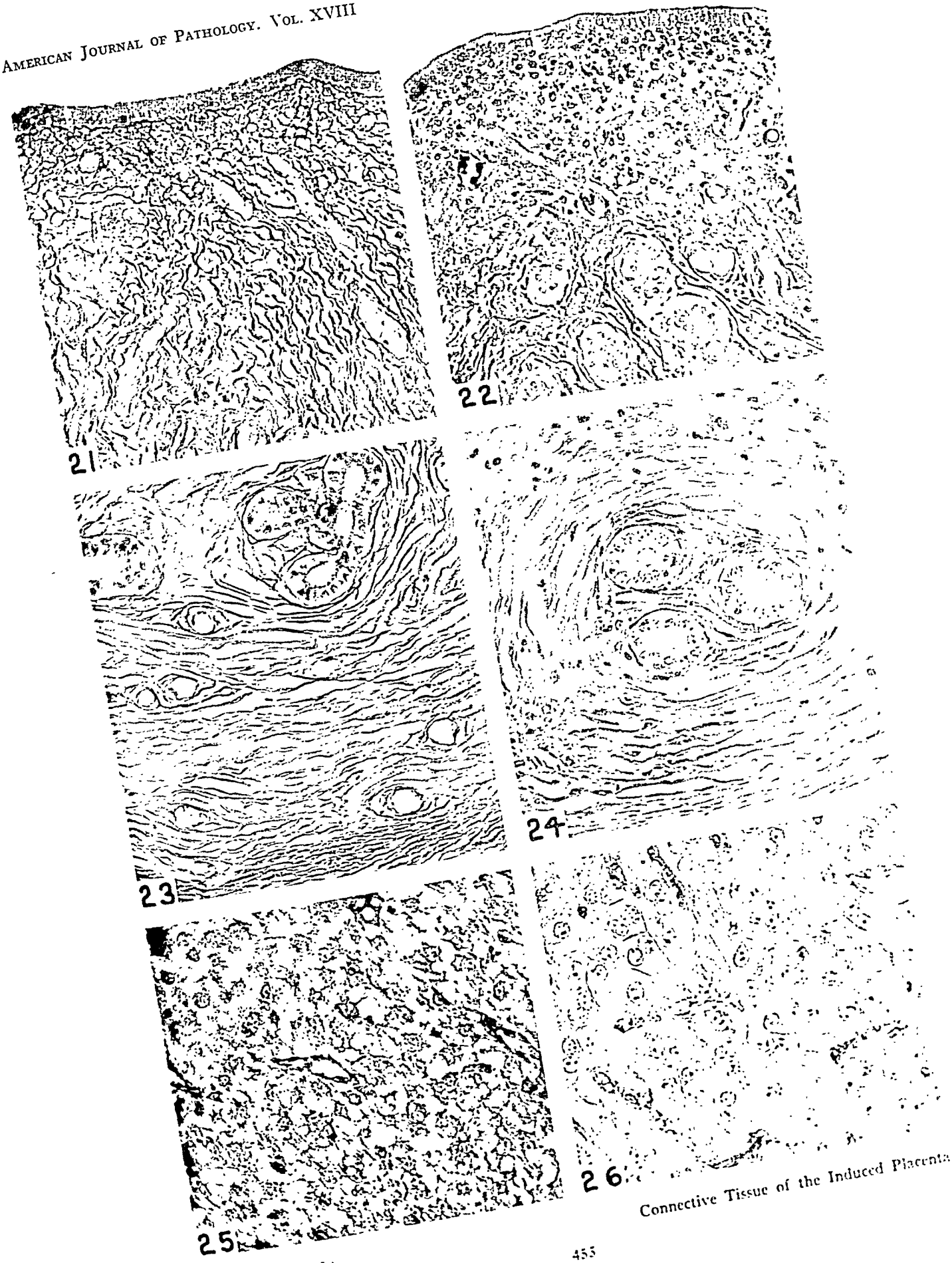
FIG. 17. Degenerating deciduoma showing large and small cells. There is a hemorrhage and a necrotic mass immediately under the lumen. Mallory preparation. × 115.

FIG. 18. Small 3-day deciduoma. Blending of the fibrillar material of the decidual growth can be seen, which is located mesometrially with that of the adjoining endometrium. Gömöri preparation. × 62.

FIG. 19. Section from degenerating deciduoma. Irregular collagenous fibers can be seen (see arrows) lying among the cellular débris. Gömöri preparation. × 720.

FIG. 20. Section from a 5-day deciduoma showing the junction of the decidual mass with the endometrium. The changes shown here may be compared with Figure 24. Mallory preparation. × 62.

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PLATE 72

FIG. 21. Normal endometrium showing the lining epithelium, the basement membrane and the subepithelial reticular zone. The reticulum gradually blends into the underlying collagen, which appears gray. Gömöri preparation. × 168.

FIG. 22. Normal endometrium showing the lining epithelium and the abundant stromal cells in the subepithelial reticular zone. There is thickening of fibers deeper in the endometrium. Mallory preparation. × 168.

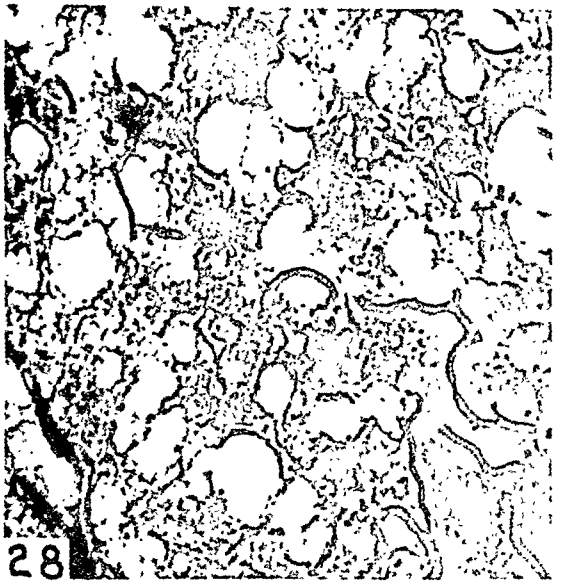
FIG. 23. Outer portion of normal endometrium lying next to circular smooth muscle. Note thickness of collagenous fibers lying parallel to the muscle and around the glands. Compare with Figure 21. Gömöri preparation. × 168.

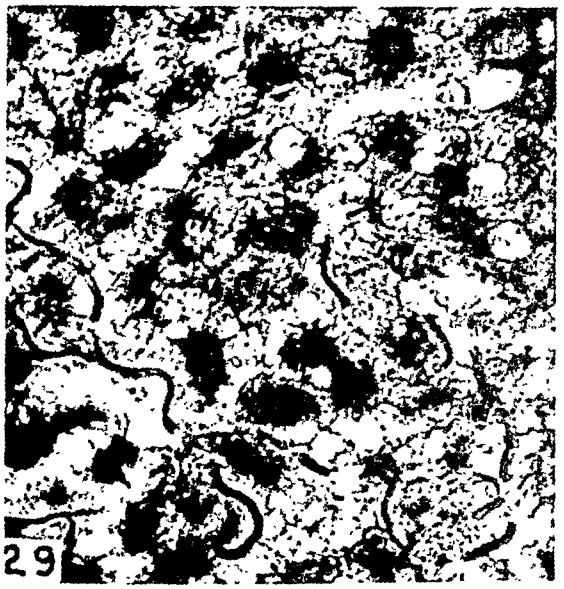
FIG. 24. Outer portion of normal endometrium adjoining the circular smooth muscle. Note the broad fibers and relative inabundance of stromal cells. Compare with Figure 22. Mallory preparation. × 168.

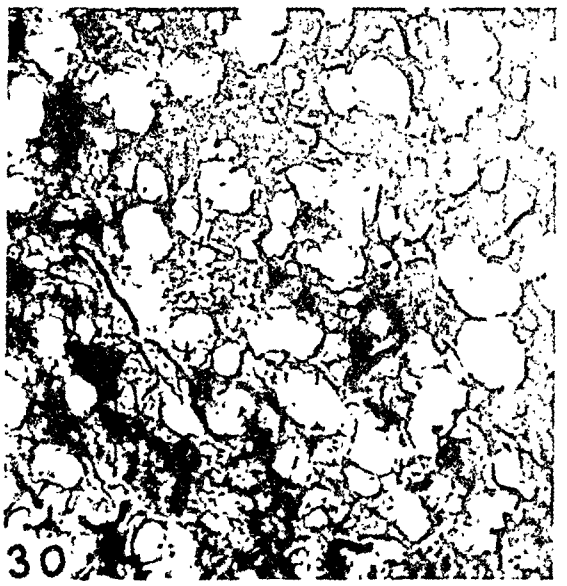
FIG. 25. Small decidual cells. Mallory preparation. × 280.

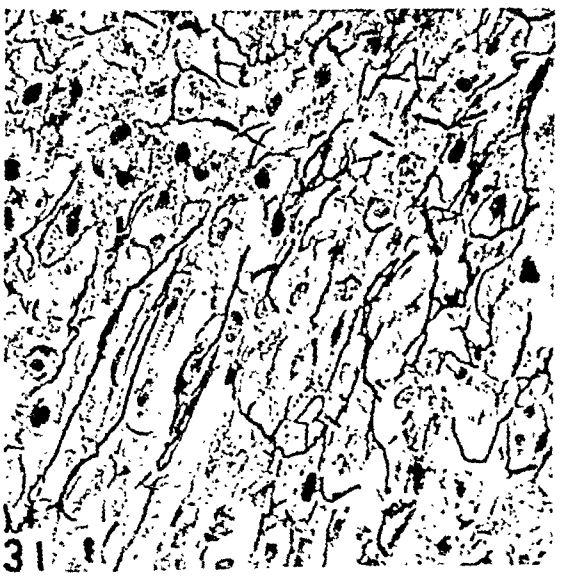
FIG. 26. Large decidual cells. Mallory preparation. × 280.


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PLATE 73

(All figures taken from Gömöri preparations.)

FIG. 27. Section from small-decidual-cell area showing a very regular reticular meshwork. × 600.

FIG. 28. Section from small-decidual-cell area showing intercellular spaces and fine reticular fibrils closely applied to the cells. There are also thick fibers which are reticular. × 600.

FIG. 29. Section from small-decidual-cell area showing smaller intercellular spaces and less abundant reticulum. Here there are the thicker fibers. × 600.

FIG. 30. Section from near periphery of small-decidual-cell areas showing an irregular arrangement of cells, intercellular spaces and fibrillar material. × 600.

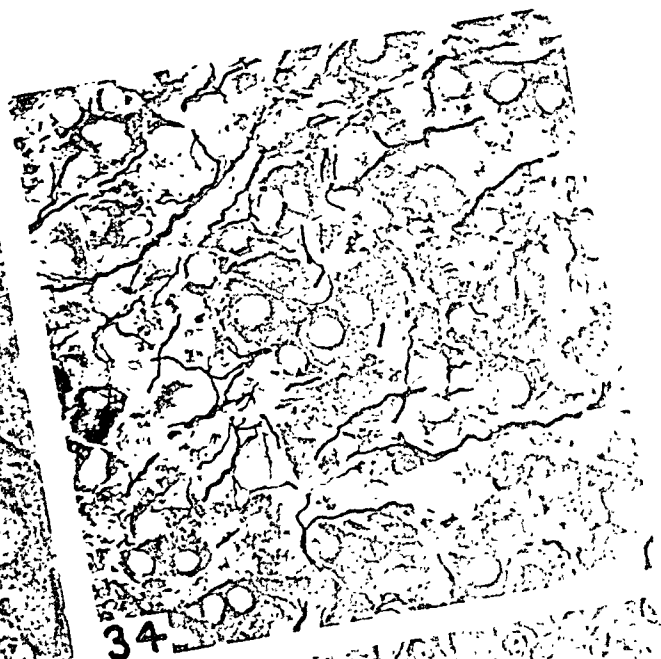
FIG. 31. Section from near periphery of small-decidual-cell areas. There are many branching fibers and fibrils, nearly all of which were reticular. × 280.

FIG. 32. Section from near periphery of small-decidual-cell areas showing an irregular arrangement of fibrillar material; the thicker fibers were transitional. × 280.

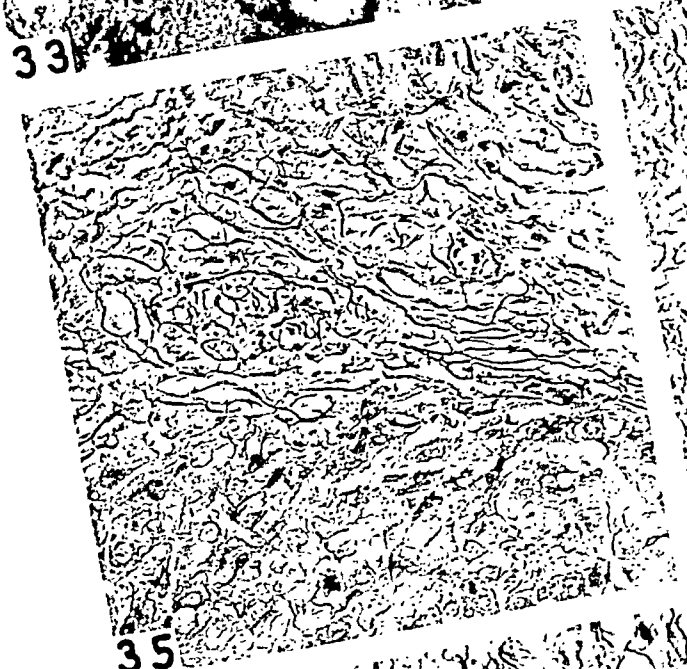
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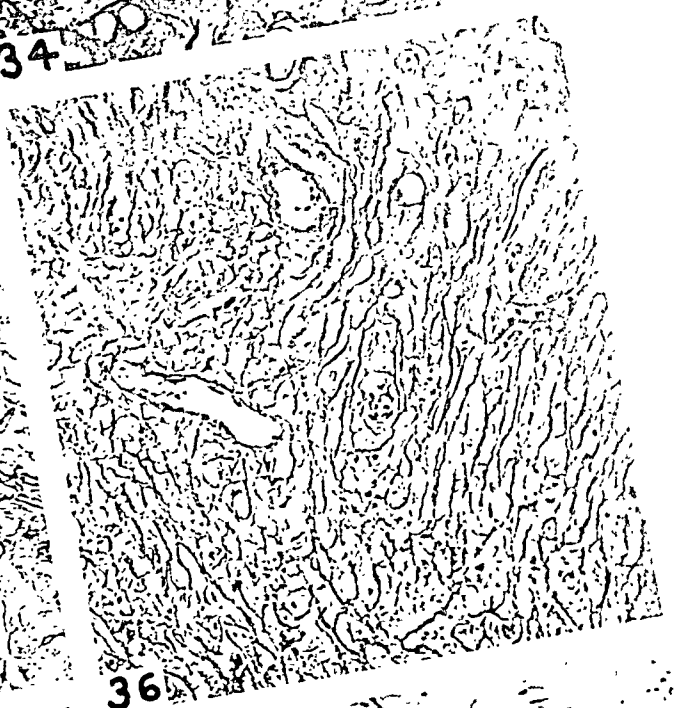
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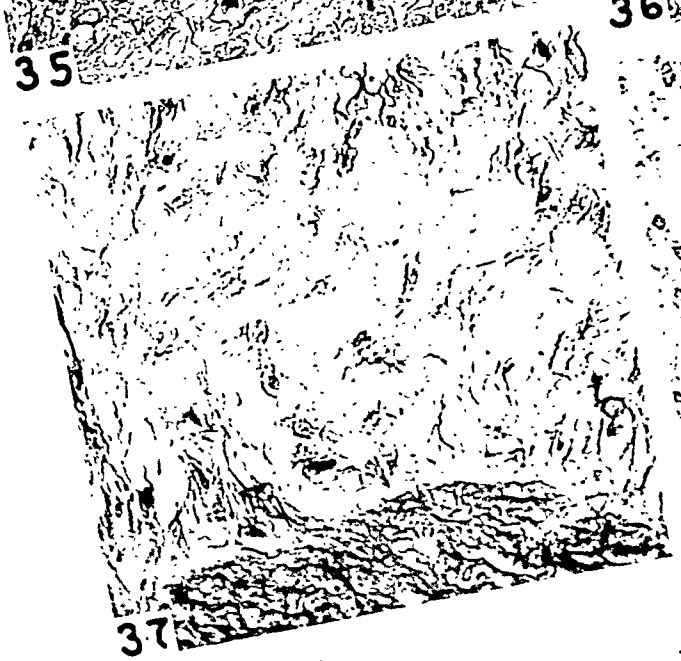
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38

Connective

4 Placenta

Wolfe and Wright

PLATE 74

(All figures are from Gömöri preparations unless otherwise noted.)

FIG. 33. Section from large-decidual-cell area showing scant and irregularly arranged fibrillar material which was reticular. × 600.

FIG. 34. Large decidual cells showing abundant fibrillar material which was reticular. × 280.

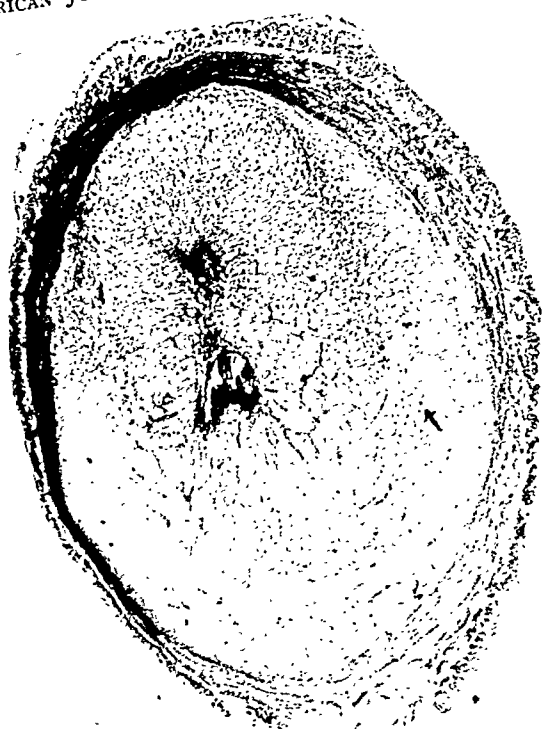
FIG. 35. Junction of a 5-day deciduoma with normal endometrium. × 96.

FIG. 36. Junction of a 3-day deciduoma with normal endometrium showing very gradual transition and broad collagenous fibers extending into the deciduoma. × 96.

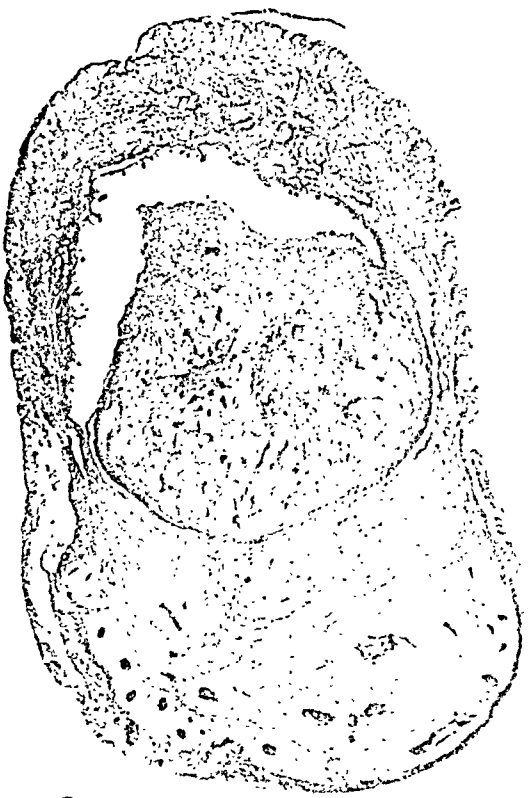
FIG. 37. Section from the outer portion of the endometrium adjoining a deciduoma. Here the thick collagenous fibers, such as those seen in Figure 23, have been replaced by a finely fibrillated collagen. Stromal cells lie imbedded in the collagenous tissue. The circular smooth muscle is shown. × 168.

FIG. 38. A Mallory preparation taken from an area comparable to that in Figure 37. The finely fibrillated nature of the collagen is evident. The darker and more compact areas represent persisting, thicker, collagenous fibers. The circular smooth muscle also is shown. × 168.

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40



41



42

Wolfe and Wright

Connective Tissue of the Induced Placenta

PLATE 75

FIG. 39. Section through a 5-day deciduoma. The small- and large-decidual-cell areas may be recognized at the mesometrial and antimesometrial poles, respectively. There is a blood-filled space near the center. Mallory preparation. × 16.

FIG. 40. Section through a 10-day deciduoma showing degenerative changes. The large-cell portion of the decidual mass has disappeared and part of the small-cell area has been replaced by a structureless mass. The uterine cavity has reappeared and is lined by epithelium on the antimesometrial side. The mesometrial region may be compared with that in Figure 39. Mallory preparation. × 16.

FIG. 41. Section through the peripheral portion of a weak 3-day decidual reaction. There is moderate hypertrophy of the stromal cells in the subepithelial reticular zone and the process is progressing into the deeper endometrium. There is reticulum in areas where stromal cell hypertrophy has occurred. This reaction occurred at the mesometrial pole. Gömöri preparation. × 181.

FIG. 42. A portion of one lobule found in the mesometrial enlargement (metrial gland). The central blood vessel is surrounded by collagen while reticular fibers surround the large granular cells. Gömöri preparation. × 181.

EXPERIMENTAL BLASTOMYCOSIS IN MICE *

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In previous papers I have shown that the virulence, for mice, of the causative organisms from two cases of human blastomycosis was not enhanced by mouse passage,[1] and that the filamentous form was as infectious for mice as the yeast form.[2]

Since blastomycosis was being produced very consistently in this convenient laboratory animal, observations were made on the reactions of the tissues of the mouse to this large fungous organism. This study was augmented with an attempt to explain some of the tissue responses by noting the results of injections into mice of killed blastomycetes and of a phosphatide fraction of the fungus. Rabbits were utilized for an accompanying study of the effect of a polysaccharide fraction on the tissues. The chemical fractions were obtained from Peck and Hauser, whose published papers about the fractions may be consulted.[3,4]

LITERATURE

In all of these reports the injected organisms were obtained from proved human cases of blastomycosis as recently tabulated by Martin and Smith.[5]

Bowen and Wolbach [6] inoculated four mice intraperitoneally and obtained satisfactory abdominal and pulmonary lesions. They stated: "The type of lesion in the lung, the filling of the alveoli with large cells and organisms with little other reaction, is a peculiar one and deserves further study." Photographs show sections of the lesions of the lung of a mouse killed 40 days after intraperitoneal inoculation. The abdominal lesions had largely disappeared. DeMonbreun [7] found, from injection of yeastlike forms of the organisms, that "each of six mice died in from three to five weeks after inoculation, and numerous small abscesses containing the fungous cells were found in the lungs, liver, spleen and kidneys." In mice inoculated by Bergstrom, Nugent and Snider,[8] profuse lesions developed within 5 weeks, but the animals did not die until 10 weeks after inoculation. Davis [9] found that blastomycetes injected into the peritoneal cavity of guinea pigs were rapidly taken up by leukocytes, macrophages principally.

* Aided by the John and Mary R. Markle Foundation and the Duke University Research Fund.

Presented before the American Society for Experimental Pathology, Chicago, Ill., April 18, 1941.

Received for publication, September 4, 1941.

TABLE I

The Cellular and Tissue Response to the Injection of Living Blastomyces dermatitidis into the Peritoneal Cavities of Mice*

Duration	No. mice	No. died	No. sacrificed	Peritoneal fluid						Gross
				White cells	Neutrophils	Undifferentiated cells	Monocytes	Eosinophils	Organisms	
				per cmm.	%	%	%	%		
½ to 3 hrs.	2	0	2	6,000	19	70	11		Free	Normal
4 hrs.	2	0	2	9,300	83	14	3		Surrounded by neutrophils	Normal
1 day	2	0	2	19,600	56	17	24	3	Surrounded by neutrophils	Normal
3, 4, 5 days	3	1	2	9,500	27	52	21		Surrounded by neutrophils and monocytes; fibroblasts present	Dull peritoneum
1 to 5 wks.	59	50	9	5,000†	54	16	29	1	Surrounded by neutrophils and monocytes; blastomycetes rare	Nodules on peritoneum and usually in lungs
5 to 8 wks.	4	2	2							Nodules on peritoneum and usually in lungs
Total	72	53	19							

* Dose varied from: 0.5 cc. of 1:200 to 1 cc. of 1:25 suspension by volume, or 0.1 to 2 mg. dry weight.
† Average of 3 mice: 8, 10, 11 days.

Materials and Methods

Two strains of *Blastomyces dermatitidis* were employed, the sources of which have been previously detailed.[1] Strain A was from a fatal case of systemic blastomycosis and strain B was from a cutaneous lesion which responded well to iodine therapy. Both strains were found to be capable of producing generalized blastomycosis in mice, and they are not differentiated in Table I.

The mice were inoculated intraperitoneally under rigid, aseptic technic with doses of pure cultures which varied from 0.5 cc. of a 1:200 suspension to 1 cc. of a 1:25 suspension, by volume. The yeast form of the organism was centrifuged in a calibrated tube at a standard speed and duration of time. Proper dilutions were made by adding saline solution. To obtain data to compare with the amounts of dead organisms and chemical fractions, the wet centrifuged blastomycetes were weighed, after the supernatant fluid was decanted. Another weighing after vacuum drying indicated that 1 cc. of the moist organism was equivalent to 52 mg. in the dry state. On this basis the dry weight injected varied from 0.1 to 2 mg. in different animals. The smaller dose was capable of producing generalized lesions, and no separation of the animals according to dosage has been made. Comment and data on the dosage factor are available in another report.[1]

Studies of the peritoneal fluid were made in mice sacrificed during the first 11 days following injection. The normal mouse has insufficient peritoneal fluid for cellular studies. Consequently, 0.5 cc. of physiologic saline solution was injected intraperitoneally just before the animal was killed. Total white counts were determined. The disposition of the blastomycetes and the comparative numbers of the cell types were determined by supravital study with neutral red and janus green, in a hot box, and by stained smears. Paraffin sections were found helpful in differentiating cell types, and gave topographic relations.

Thomas and Dessau [10] studied the cells of the peritoneal fluid in normal mice after rinsing the peritoneal cavity with 0.5 cc. of saline solution as just mentioned. The total cells averaged 5,900 per cmm. Undifferentiated cells made up 65.5 per cent in the differential counts. These "were small cells, about the size of the blood lymphocytes, with a small amount of cytoplasm and no constant cytoplasmic inclusions which stained with vital dyes." Monocytes numbered 32 per cent; polynuclear leukocytes, 1.5 per cent; basophilic leukocytes, 0.8 per cent, and plasmatocytes, 0.2 per cent. This classification has been followed in these experiments.

thought to be predominantly injured polymorphonuclear neutrophils, though some were monocytes. Fibroblasts extending from such a mass at the end of 8 days are shown in Figure 2.

At 1 week white nodules and plaques were visible over the peritoneal and omental surfaces, and these continued to increase in size.

The gross appearance typical of the period between 1 and 5 weeks has been shown in previous papers.[1,2] Often a single, large, caseous nodule occurred between stomach and spleen, and smaller nodules elsewhere. At 1 week blastomycetes had been first noted in the retrosternal lymph nodes and in the lungs. From then on involvement of the lung was usually prominent and was noted in 90 per cent of the animals which died in this period (Fig. 5; also Fig. 4 in previous paper [2]). The route from the peritoneal cavity to the lungs was never by direct extension through the diaphragm because the lungs were free in the pleural cavities. The uniform distribution of nodules in the lungs indicated an hematogenous spread, either via the retrosternal lymphatics and nodes to the venous system, or directly from the peritoneal cavity into the vascular system. Hematogenous spread to cardiac muscle (Fig. 5 in previous paper [2]), brain, spleen and liver was demonstrated microscopically in a number of mice, and probably would have been demonstrated much more frequently if all of these organs had been systematically studied microscopically. An interesting involvement of the liver consisted of growth of organisms into portal veins with the production of large areas of infarction.

Microscopically, this period was characterized by the great proliferation of blastomycetes, whether in the abdominal cavity, lungs (Fig. 6) or elsewhere. There was little reaction of any kind in some masses but usually the blastomycetes were interspersed with polymorphonuclear cells (Fig. 6), fragments of nuclei, or less frequently with monocytes, and very rarely with the undifferentiated cells. Necrosis of the centers of blastomycetic masses was the rule; and fibroblastic growth developed at the periphery after a time, but generally the cellular reaction was less conspicuous than the blastomycetic proliferation.

Microscopic abscesses like those of human cutaneous blastomycosis rarely occurred. This may have been due to the fact that the blastomycetic growth was so massive and diffuse that microscopic collections of a few blastomycetes with surrounding polymorphonuclear cells were not frequent. Figure 4 shows a microscopic "abscess" adjacent to the pancreas in a mouse injected 11 days before.

Phagocytosis of blastomycetes by macrophages and giant cells was never a frequent appearance but it did occur, especially in the animals which had been injected for the longer intervals. Several giant cells

Experimental Data

Living blastomycetes were injected into the peritoneal cavities of 72 mice. The great majority of mice died between 1 and 5 weeks after inoculation (Table I) and presented peritoneal and pulmonary nodules, and usually microscopic lesions elsewhere. Most of the mice which died became less active and lost weight during the several days before death.

During the first 3 hours the injected blastomycetes lay free in the abdominal cavity and the white and differential counts were those of normal mice. After several hours (Fig. 1) the blastomycetes were found surrounded by polymorphonuclear neutrophils. The peritoneal fluid also showed an increase in the number of white cells and polymorphonuclear neutrophils predominated instead of undifferentiated cells.

At the end of 1 day the organisms were surrounded by cells which were difficult to identify. In the supravital preparations the cytoplasm contained numerous, neutral-red vacuoles, and nuclear staining sometimes occurred, which, in such preparations, indicates dead cells. The nucleus was swollen and usually was not polymorphous. In the stained films the cytoplasm was like that of neutrophils. Study of paraffin sections suggested that most of these surrounding cells were probably injured polymorphonuclear neutrophils with swollen nuclei. The same difficulty was experienced in interpreting the nature of the surrounding cells throughout the 11-day period during which observations of peritoneal fluid were made. No such difficulty was experienced in making differential counts of the peritoneal fluid. The total white count was definitely elevated and the polymorphonuclear cells predominated in the differential count. The coating on the peritoneum was found in paraffin sections to consist of blastomycetes, interspersed with polymorphonuclear neutrophils.

In the period from 3 to 5 days the blastomycetes were surrounded by cells which were judged to be polymorphonuclear neutrophils, but some stimulated monocytes and occasional fibroblasts were noted. The peritoneal surfaces were dull to the naked eye, and occasional unattached white nodules 1 mm. in diameter were seen. At 5 days (Fig. 3) the peritoneal coating of blastomycetes and interspersed polymorphonuclears had become necrotic centrally in the nodules, but peripherally the blastomycetes were proliferating and attracting polymorphonuclear neutrophils. Karyorrhexis among dead polymorphonuclear neutrophils was often noted. Monocytes were present but not prominent. Fibroblastic outgrowth from the serosa was well developed and vascularization had begun.

In the period from 1 to 5 weeks, free blastomycetes in the peritoneal fluid were less numerous. They occurred in a mass of cells which were

every other day. (Dosage is in terms of equivalent dried weight of suspension.) Killing was effected by heating at 60° C. for 2 hours. After each aspiration of suspension from the rubber-stoppered vaccine bottle, the heating was repeated to insure sterility.

The mice tolerated the large doses poorly, and appeared ill after injections. Most of them died in 5 to 11 days, apparently of the toxic effects of the suspension.

In paraffin sections the fact that the fungus was dead was clearly evident in the failure of the blastomycetes, especially the internal substance, to stain with hematoxylin. Uniform staining with eosin occurred.

The peritoneum was lined with a friable, yellow layer. This was composed largely of the deposit of killed blastomycetes (Fig. 7). Between them, as between the living organisms in the first experiments, polymorphonuclear cells and a few mononuclear cells occurred. There was the same necrosis in the centers of large blastomycotic masses as was noted with the living organisms; but with the killed blastomycetes the necrosis applied only to the infiltrating cells, since the blastomycetes themselves were already dead. Eosinophils, and macrophages and giant cells containing blastomycetes, were more abundant than in the mice which received the living organisms. In the mouse examined 11 days after injection the outlines of the blastomycetes had largely disappeared, leaving "caseous" areas.

Pulmonary lesions were not produced. The retrosternal lymph nodes contained very large, finely granular reticulo-endothelial cells, but no blastomycetes.

In comparison with the living organism it was concluded that the initial cellular response, the central necrotizing process and the peripheral, fibroblastic response were similar. The early toxicity of the large doses of killed organisms was correlated with the toxicity of the living organisms after several weeks. By this time the formerly viable masses had become necrotic centrally and had probably liberated some toxic substance in the nature of an autolysate. Enzymes from the dead polymorphonuclear neutrophils may have produced the necrosis.

Blastomycetic Phosphatide in Mice

For the method of extraction and an account of the properties of this phosphatide the paper by Peck and Hauser [3] may be consulted.

Thomas and Dessau,[10] who studied the effects of chemical fractions of the tubercle bacillus in mice, stated: "It is of interest that while in guinea pigs and in rabbits the reaction to tuberculo-phosphatide resembles very closely the lesions produced by the living organisms, this mimicry is best accomplished in the mouse by the injection of the waxy

containing blastomycetes were noted in a mouse injected 34 days before.

In the few mice examined in the interval between 5 and 8 weeks, the central areas of blastomycotic masses were not only necrotic, but the outlines of the blastomycetic shells had disappeared, so that the appearance of caseation was produced. Dense fibrosis occurred about such areas.

"Toxicity" of Process

Some of the sacrificed animals were active and appeared healthy, but showed extensive abdominal and pulmonary lesions. It is not clear, therefore, that blastomycetic infection in itself is especially productive of a toxic state. The same may be said for human cases of blastomycosis, except in the terminal stages of the process. As possible causes for death in the mouse, aside from a toxic effect of the organism or of the products of disintegration of the lesions, are the presence of blastomycosis of the peritoneum which may interfere with peristalsis of the intestines, or blastomycosis of the liver which may interfere with the function of that organ. The arguments in favor of the toxicity of dead organisms will become apparent in the following section on killed blastomycetes.

Summary of Changes

The intraperitoneal inoculation of mice with *B. dermatitidis* led to a massive growth of organisms in the peritoneum with dissemination to the lungs and elsewhere. The blastomycetes attracted cells, chiefly polymorphonuclear neutrophils, and when the organisms occurred in masses they tended to develop necrotic centers. The early, peripheral, fibroblastic growth developed into an encapsulating, fibrous layer. The monocyte and giant cell appeared to be less important in the mouse than the polymorphonuclear neutrophil and the fibroblast.

Injection of Killed Blastomycetes

Killed blastomycetes were injected into the peritoneal cavities of 14 mice to determine, first, how closely the tissue response to the living organisms could be duplicated, and second, how toxic the suspension was.

A single small dose of killed organisms produced no apparent effect. Mice which had received 0.1 mg. were sacrificed after 8 weeks. No gross or microscopic abnormality could be demonstrated. It has already been shown that this amount of living fungus produced progressive blastomycosis and death.

To duplicate the effects of the proliferating, living yeast cells, repeated large doses of killed organisms were used. Ten mg. were given

water there were produced sterile peritonitis, retrosternal lymphadenitis and the impressive changes in cells of the peripheral blood stream shown in Chart 1. These changes in the white blood cells consisted of (1) almost immediate leukopenia, (2) lymphopenia reaching the lowest point in 5 or 6 hours and (3) a great increase in immature polymorphonuclears (amphophils) which reached the maximum in 5 or 6 hours. The curve of these immature, non-filamented polymorphonuclears in Chart 1 ascends and crosses the curve of the more mature, filamented polymorphonuclears. This is thought to indicate that the bone marrow was stimulated to send these immature forms into the blood stream.

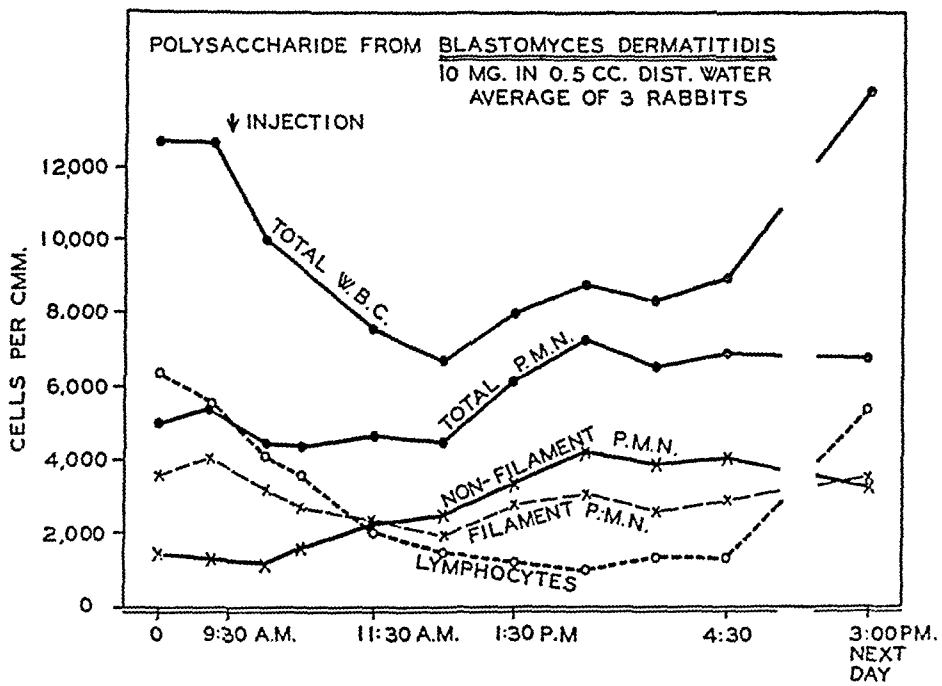


Chart 1. Changes in the number of white cells in the blood streams of three rabbits which received a polysaccharide of *Blastomyces dermatitidis* intraperitoneally.

The transient nature of the changes in the levels of the blood cells is indicated in Chart 1. They tended to return to the starting points on the second day when the rabbits were killed. Each of the individual graphs from which this average graph was constructed showed the same trend in lymphocytes and in non-filamented (immature) amphophils. The curve of the non-filamented forms of each rabbit crossed and went above the curve of the filamented forms.

Control studies in 13 rabbits injected intraperitoneally with distilled water, dextrose, saline solution, barium sulfate and lamp black, indicated that the described reactions in the blood stream were not specific for the blastomycetic polysaccharide, but could be elicited by other substances. However, the degree of change was greater with the blastomycetic polysaccharide.

fractions." They found that the phosphatides of the tubercle bacillus stimulated the multiplication and partial maturation of monocytes. The formation of true epithelioid cells was not the rule.

The blastomycetic phosphatide had been passed through a Berkefeld filter for sterilization when it was in the stage of ether extraction. For injection of mice, 10 mg. were suspended in 1 cc. of distilled water. Microscopically the suspension showed droplets of which none was as large as a human red blood cell. After the suspension stood for a day, a small amount of material settled out.

Seven mice received the phosphatide intraperitoneally, in amounts varying from a single dose of 10 mg. to daily doses of 30 mg. for 24 days, with sacrifice 3 days later. The higher dosage resulted in prominent, white, peritoneal plaques.

A peritoneal reaction, predominantly of the monocytic series of cells, was seen in sections. Most of these cells were of moderate size. A few had fine vacuoles, some of which, in frozen sections, absorbed a little scharlach R. In addition to the predominant "large mononuclear cell," polymorphonuclear neutrophils and eosinophils, fibroblasts and reticulum were occasionally noted, the latter in mice allowed to survive for the longer periods.

Apparently, then, the phosphatide of the blastomycete acts on the mouse in much the same manner as does the phosphatide of the tubercle bacillus.

The significant point here is that the large majority of reacting cells were of the monocytic series (Fig. 8) rather than of the polymorphonuclear series. Moreover, a layer of the same thickness caused by the living or killed blastomycetes would show necrosis centrally, as was demonstrated by direct comparisons. Hence the phosphatide is not the chemical fraction responsible for the polymorphonuclear reaction and the necrosis, the two most striking features in connection with the living or killed organisms.

Further studies with chemical fractions have not been pursued in the mouse.

Blastomycetic Polysaccharide in Rabbits

A polysaccharide from *B. dermatitidis* was injected intraperitoneally into rabbits, because repeated samples of blood are easier to obtain in rabbits than in mice, and because a report on the effects of polysaccharides of certain bacteria on rabbits was already available for comparison.[11] Preparation 1 as described by Peck, Martin and Hauser [4] was used.

During the first few hours after the injection of 10 mg. in distilled

Heat-killed and living blastomycetes provoked similar cellular responses in the peritoneal cavities of mice.

3. Blastomycetic phosphatide repeatedly injected intraperitoneally into mice caused cells of the monocytic series to respond. This fraction is therefore not responsible for the polymorphonuclear response and the necrotizing effect related to the living organisms.

4. In rabbits, single intraperitoneal injections of blastomycetic polysaccharide produced, in the first few hours, sterile peritonitis, retrosternal lymphadenitis and remarkable changes in the blood stream consisting of leukopenia, lymphopenia and increase in the numbers of immature amphophils. These changes are similar to those which have been produced by polysaccharides from tubercle bacilli and pneumococci, as described by Sabin, Joyner and Smithburn.

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Comment could be made on the microscopic character of the peritoneal exudates, the retrosternal nodes and the spleens. However, nothing additional to what has already been reported [11] concerning phagocytosis of polymorphonuclear neutrophils and peritoneal reaction was learned, and details are omitted.

It is apparent that these experimental results are very similar to those obtained from the injection of the tuberculo-polysaccharide as reported by Sabin, Joyner and Smithburn.[11] The increase in the immature polymorphonuclear cells and the decrease in the lymphocytes of the blood stream occur as strikingly after the injection of blastomycetic polysaccharide as they do after the injection of the polysaccharides which these authors employed.

Without the employment of numerous control polysaccharides derived from many sources it would be difficult to maintain that the polysaccharides so far employed have any highly specific effect. The control substances have been dissimilar with respect to molecular weight and solubility.

Of greatest general interest is the information, not new, but confirmed, that exceedingly minute quantities of various substances injected intraperitoneally produce not only the mild, acute peritonitis which might be expected but also significant changes elsewhere in the body, as in the retrosternal nodes, blood stream and spleen (and bone marrow also, according to Sabin, Joyner and Smithburn [11]).

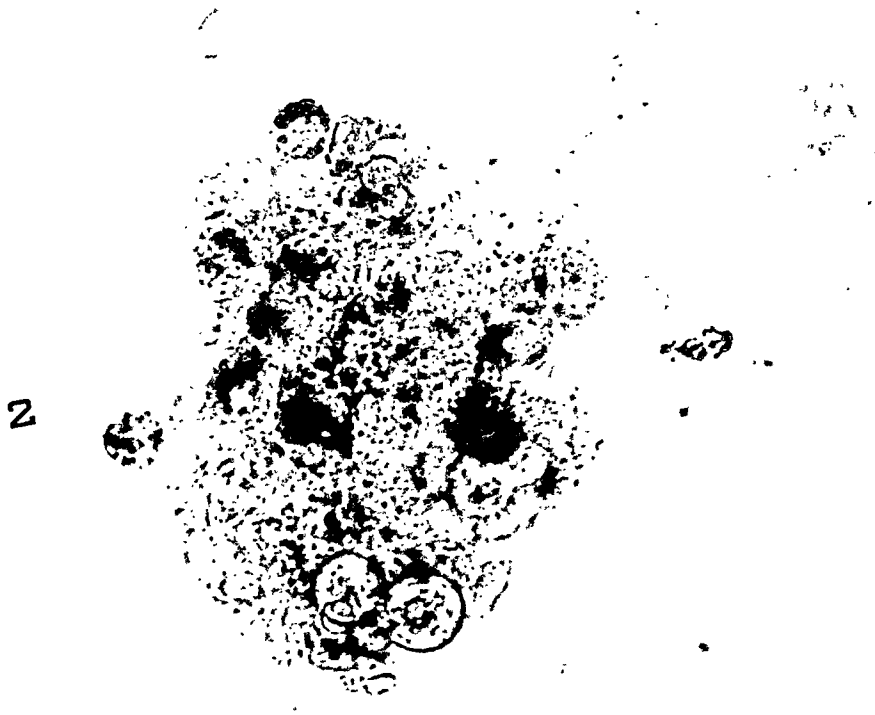
Summary and Conclusions

A study was made of the effects on mice of intraperitoneal injections of living *Blastomyces dermatitidis*, killed suspensions of the same organism and a phosphatide fraction of this fungus; also a polysaccharide fraction was studied in rabbits. The following results and conclusions are recorded:

1. The mouse was preëminently suited to the experimental production of blastomycosis. The experimental disease was characterized by the continued response of polymorphonuclear neutrophils to the luxuriant growth of the fungus throughout the infected animal and by the necrosis of the blastomycotic masses. The lesions in mice consisted mainly of organisms, whereas the lesions in most infectious diseases consist mainly of the reacting cells of the hosts.

2. Repeated intraperitoneal injections of heat-killed *B. dermatitidis* were toxic for mice, and often lethal. This is thought to explain the final lethal effect in the experimental disease, in which masses of organisms and intermingled reacting cells became necrotic, and probably permitted the absorption of substances like those associated with the suspensions of the heat-killed organisms.





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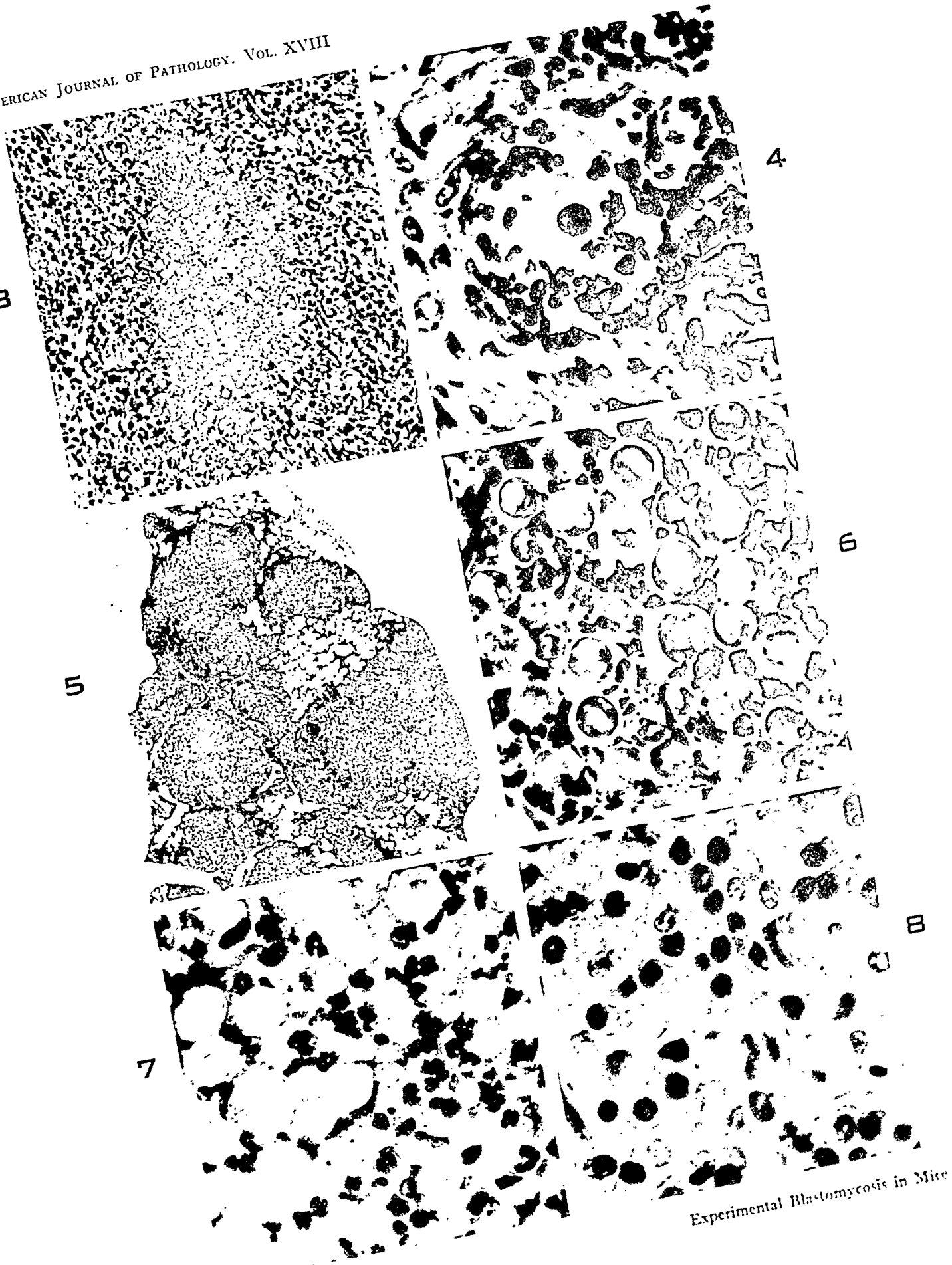
DESCRIPTION OF PLATES

PLATE 76

FIG. 1. Cellular reaction about living blastomycetes several hours after intraperitoneal injection. Peritoneal fluid of mouse. Neutral red, supravital preparation. × 715.

FIG. 2. Similar preparation, 8 days after injection. Two blastomycetes below in mass of reacting cells; fibroblasts above and to the right. × 715.

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Experimental Blastomycosis in Mice

PLATE 77

FIG. 3. Necrosis in center of peritoneal blastomycotic mass. Experimental blastomycosis in a mouse, 5 days after intraperitoneal injection. × 174.

FIG. 4. Focal lesion in peritoneum; single blastomycete surrounded by polymorphonuclear neutrophils. Mouse, 11 days after injection. × 760.

FIG. 5. Large pulmonary nodules in a mouse, 20 days after intraperitoneal injection. The pale, central areas are largely necrotic. × 14.

FIG. 6. Higher magnification of area in the preceding figure. This field is from the periphery of a pulmonary nodule where the blastomycetes are viable, as indicated by the staining of the internal substance of the blastomycete. The interstices between blastomycetes are filled with cells, chiefly polymorphonuclear neutrophils, and nuclear fragments. × 760.

FIG. 7. Cellular response in peritoneum to heat-killed blastomycetes, 5 days after initial intraperitoneal injection. There is a similarity to Figure 6, except for the lack of staining of the central portions of the blastomycetes, indicating that they are dead. × 760.

FIG. 8. Reaction to repeated injections of blastomycetic phosphatide. Cells of the monocytic series on the parietal peritoneum; fibroblasts are also present. This mouse received intraperitoneally 30 mg. of phosphatide in distilled water daily for 24 days, and was sacrificed 3 days after the last injection. × 760.

TISSUE REACTIONS IN HUMAN BLASTOMYCOSIS *

An Analysis of Tissue from Twenty-Three Cases

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In North Carolina many human cases of blastomycosis have been encountered. Reports on them have dealt primarily with the general and immunologic features of the disease,[1] or with particular features such as laboratory diagnostic,[2] gynecologic,[3] cardiac,[4] cutaneous[5] and mycologic [6] considerations. From animal experimentation in connection with these human cases it has become apparent that the mouse is preeminently suited to the experimental production of blastomycosis.[7] The comparatively abundant histologic material available has permitted a restudy of the reaction of the tissues of man to this large fungous organism. Since it has been stated that blastomycosis and tuberculosis are practically identical histopathologically,[8,9] this aspect of the reaction has been approached with especial interest.

In a comprehensive analysis of blastomycosis, Stober,[10] who based his conclusions on a large group of especially well studied cases, stated: "The changes most characteristic of blastomycosis are the cutaneous ulcerations, the deep and superficial abscesses, and the often tubercle-like nodules in the viscera." The emphasis in this quotation is on ulceration and abscess formation, although it is indicated that tubercle-like nodules occur. Others have adopted about the same view.

In more recent years the emphasis in papers on blastomycosis appears to have shifted somewhat. One might be led to believe that the tissue response in blastomycosis is identical with that in tuberculosis. Medlar [8] suggested this view in a report of two cases of pulmonary blastomycosis. D'Aunoy and Beven,[9] in a report of 26 cases, 16 of which were cutaneous, went so far as to state that "only the presence of the specific organisms either active or dead, allowed making an histological differentiation from tuberculosis in any of the cases." I supported this opinion in a case report,[3] but have now altered my view.

The observations to be reported reëmphasize the importance of abscess formation in blastomycosis, while they do not deny the occurrence, in some cases, of lesions like those commonly seen in tuberculosis. In most instances in which a differentiation was to be made by biopsy,

* Aided by the John and Mary R. Markle Foundation and the Duke University Research Fund.

Read by title at the Forty-First Annual Meeting of the American Association of Pathologists and Bacteriologists, New York City, April 11, 1941.

Received for publication, September 4, 1941.

toxicity of the patient. In histologic study a quantitative estimate was made of each of the following features: viable blastomycetes, dead blastomycetes, polymorphonuclear abscesses, giant cells and caseation.

Basis of Diagnosis

In 16 of the 23 cases, *Blastomyces dermatitidis* was recovered from lesions, and identified by cultural methods in the Department of Bacteriology. The mycologic and other criteria for identification in these cases are given by Conant and Martin.[6] The organism grew as a budding yeast at body temperature, and as a mold, showing mycelial threads and lateral conidia, at room temperature.

In all 23 cases double-contoured, yeastlike organisms, sometimes budding, were demonstrated in lesions regarded as characteristic of the disease. In my opinion, the histopathologic diagnosis of the disease, with the etiologic agent present in the lesions, is highly accurate—as accurate as the diagnosis of tuberculosis on the basis of finding acid-fast organisms in lesions characteristic of tuberculosis. Other yeastlike organisms, such as those of moniliasis, cryptococcosis (torulosis), or coccidioidal granuloma, are distinctive in sections. However, attempts should be made to obtain pure cultures in every case. Cultural methods of diagnosis, even without sections to show the characteristic tissue response, have proved to be reasonably accurate, since *B. dermatitidis* has not been shown to be a common secondary invader in human lesions or exudates. The method of direct examination of exudates or other material in 10 per cent sodium hydroxide should, of course, precede all other methods. In obscure pulmonary conditions in which blastomycosis is suspected, a skin test and complement-fixation reaction should be done.[11]

Involvement and Spread

The extent of involvement of the body was determined satisfactorily in the four cases upon which autopsies were done, as indicated in Text-Figure 1. In all four the lungs were involved. In cases 1, 2 and 3 there must have been hematogenous dissemination since lesions occurred throughout the body. In case 4 the lesions were confined to the thoracic region, and all could have extended directly from the oldest pulmonary lesion in the left lower lobe. This is the distinction between systemic and thoracic cases.

From the clinical histories it was thought that the portal of entry in the cases examined by autopsy was the respiratory tract. Therefore, thorough search was made for a primary pulmonary lesion such as occurs in tuberculosis, or for an old healed blastomycotic lesion which might have altered the susceptibility of the individual to reinfection.

the histologic appearance was thought to be highly characteristic. The presence of microscopic abscesses, certainly not common in tuberculosis, stimulated search for blastomycetes. On the other hand, it is not desirable to make the definite histopathologic diagnosis of blastomycosis without demonstration of blastomycetes.

All available clinical and pathologic data were analyzed in 23 cases of human blastomycosis from which tissue had been obtained. These occurred in the 10-year period ending in July, 1940. Twenty had been studied clinically at Duke Hospital. In 3, tissue came from other hospitals in North Carolina. Several patients were seen at Duke Hospital during the same decade from whom no biopsy had been obtained. For valuable data upon 11 of the 23 cases the reader is referred to other papers.[1,3,4]

For the present study, data were recorded in relation to the time when the tissues were obtained for examination, and the following facts

TABLE I

Human Blastomycosis, 23 Cases

Ages: 5 to 70 years

Race: Colored, 16; White, 7

Sex: Male, 20; Female, 3

Tissue available:
- Autopsies, 4
- Bone resections, 4
- Deep tissue resections, 2
- Skin biopsies or resections, 13

Involvement:
- Generalized, 7 cases
- Thoracic, 3 cases
- Skin, 13 cases (single lesions, 9 cases; multiple lesions, 4 cases)

Duration before histologic examination: 6 weeks to 13 years

Organism cultured: 16 cases

Microscopic sections:
- Viable organisms: always present
- Dead organisms: often present
- Polymorphonuclear abscesses: always present
- Giant cells: always present
- Caseation: present in generalized cases and many "deep" cases; not noted in skin cases

and observations in each case were tabulated: Race, age, sex, first manifestations of the disease, clinical course, proved or inferred involvement of the body, duration of the disease up to the time of histologic examination and outcome. The gross and histologic appearances of the lesions were studied in the light of additional data: whether iodides had been administered just before tissue was obtained, the results of skin tests and complement-fixation tests and the degree of

from bone to skin surfaces, from spine to iliopsoas abscesses, from lung to pleura, and probably from pleura to pericardium. In case 2 a tracheo-esophageal fistula had formed in connection with a blastomycotic lymph node which lay between these channels.

The cases from which specimens of bone or deep tissues had been obtained were also systemic or thoracic. There was no evidence for primary bone involvement as from penetrating wounds.

In the cutaneous group, those examples with multiple ulcers widely separated on the body and those with roentgenographic evidence of "fibrosis of the lungs" suggested the possibility that some cutaneous lesions might have originated hematogenously, perhaps from a subclinical pulmonary focus. The only alternative explanation for widely separated skin lesions is auto-inoculation.

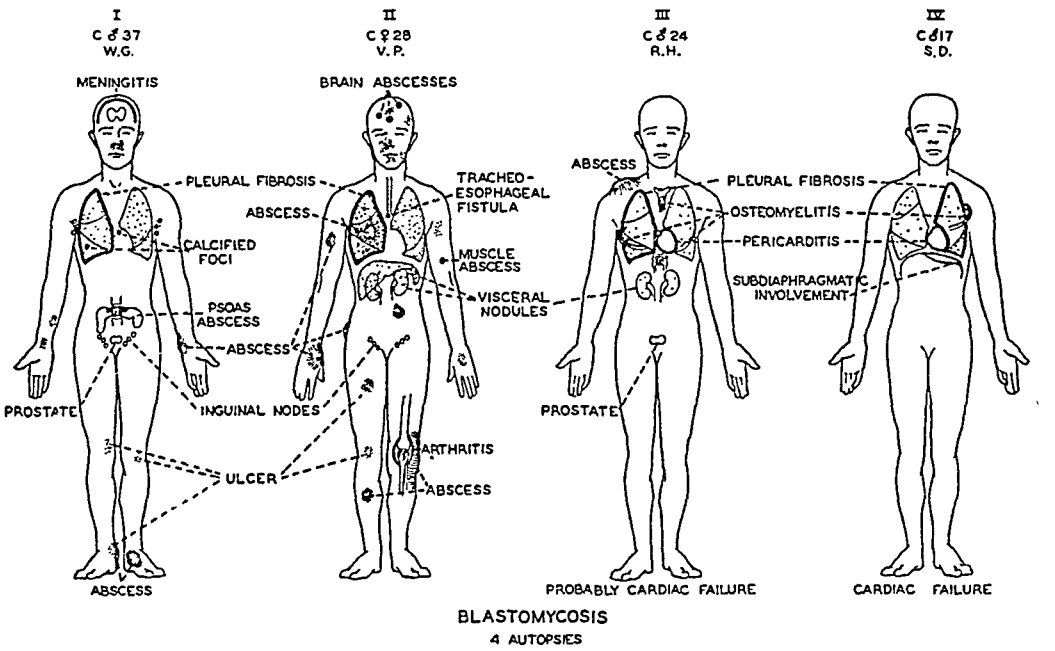
Most of the cases with cutaneous involvement were thought to be primary, probably from direct inoculation, as in wound infection, although precise information on this point was unobtainable. Cases are on record of cutaneous blastomycosis following traumatic injuries,[12] and cutaneous blastomycosis can be produced in monkeys by intracutaneous injections.[13] In most of our cases spread from single cutaneous lesions appeared to be by peripheral extension, either direct or by the cutaneous lymphatics. The cutaneous ulcerations found in the patients of autopsies no. 1 and 2 seemed to be of hematogenous origin.

Toxicity

A close correlation between toxicity and systemic or deep involvement was noted. Most of the patients with cutaneous lesions were ambulatory and "non-toxic." All of those who came to autopsy were judged to be 4 plus (++++) in respect to toxicity, on the basis of temperature, blood-cell studies and weakness. In three, death appeared to be due directly to blastomycotic involvement; in one, to heart failure secondary to constrictive blastomycotic pericarditis (case 4). An element of heart failure may have been present in case 3 also, and on the same basis.[4]

Study of the tissues suggested that the toxicity of the patient was related to two features: (1) large numbers of organisms and (2) caseation. In material obtained by autopsy and from deep lesions, organisms were often present in enormous masses, almost in pure culture, as in the brain abscess (Figs. 2 and 9) of autopsy no. 2. Organisms were often dead in the central portions of such masses, reminiscent of the findings in experimental blastomycosis in mice. In the cutaneous cases, on the other hand, organisms occurred singly or in pairs (*Fig.* 1) and the lesion was composed of cellular (polymorphonuclear) exudate, or granulation tissue and hyperplastic epithelium.

An "oldest" lesion was identified in cases 2, 3 and 4. In case 2 this was a ragged abscess cavity in the right middle lobe (Fig. 8); in case 3, blastomycotic scarring of the upper portion of the right lower lobe; and in case 4, blastomycotic scarring at the base of the left lung. In case 1 calcified foci were found in the right lower lobe and in the draining hilar node, which were as likely to be tuberculous as blastomycotic,



Text-Figure 1. The location of lesions in four cases of blastomycosis studied at autopsy.

if not more so. No organisms were demonstrable in the calcified lesions. Dense pleural fibrosis occurred in each case on the side which appeared to be primarily affected.

Thus in the four cases coming to autopsy there was no close analogy to adult or reinfection tuberculosis. The process began in one portion of the lungs, developed there for a time, and then spread to the rest of the lungs and usually to the general circulation. No stage of massive mediastinal blastomycotic lymphadenitis occurred, analogous to childhood or first-infection tuberculosis.

Lymphatic spread was noted as from lesions on the extremities to axillary or inguinal nodes, and from lungs to peribronchial nodes. Hence the lymphatic system was not immune to blastomycosis. The thoracic duct appeared to be normal, grossly and microscopically, in three cases. This does not prove that transmission of organisms cannot occur by this duct, but suggests that lymphangitis is not common in blastomycosis, in contrast to sporotrichosis.

Direct extension was a frequent and important method of spread:

Fundamental Microscopic Characteristics

In every case the polymorphonuclear neutrophil was a prominent cell type (Figs. 1 to 5) and usually it occurred in abscesses. In two cases with deep lesions, true abscesses were not present and the polymorphonuclear reaction was spoken of as diffuse. Sometimes the polymorphonuclear neutrophils occurred in the interstices between blastomycetes, as in the massive lesions of the generalized type; and sometimes the opposite relationship existed, *i.e.*, one or two blastomycetes occurred in a mass of pus, as in the sparser involvement of the cutaneous cases. The former type of involvement is well illustrated by Mallory.[15] Eosinophils were occasionally prominent. Giant cells (Fig. 5) were noted in every case, but were sometimes very rare. Usually blastomycetes, either living or dead, occurred in giant cells, but in a few instances no organisms were noted. Large mononuclear cells were not prominent.

Caseation was not found in the lesions of the solely cutaneous cases, nor in the skin lesions of the cases coming to autopsy. In fact, the skin lesions of the latter group closely resembled those of the "pure" skin cases. Abundant caseation was noted in autopsies nos. 1, 3 (Fig. 6) and 4, and in all the cases with osseous and deep lesions except two. In specimens obtained at autopsy it could sometimes be determined, from the presence of shadows of dead blastomycetes, that caseous material was composed largely of dead fungi. This was true of lesions of the heart, brain, prostate and bone. In some of the caseous pulmonary lesions, on the other hand, no shadows were noted in the caseous material. It was not evident whether the necrosis was due to proteolytic enzymes of the contained polymorphonuclear neutrophils, or to the poor nutrition of those blastomycetes which lay centrally, or to other factors.

Fibrosis was frequently prominent (Fig. 7). In many lesions fibrosis about abscesses had developed a heavy collagenous component.

Viable organisms were always present, free or within giant cells. Dead organisms, free or within giant cells, were present in material obtained by autopsy, and in most of the cutaneous lesions. The differentiation between viable and dead organisms was made on the basis of staining reaction. Blastomycetes appearing as shadows were obviously dead. Forms in which the central portion of the organism no longer absorbed any hematoxylin were also considered dead. The validity of this differentiation was supported by the appearance, in sections, of the heat-killed blastomycetes injected experimentally into mice.

From this analysis, it is clear that the microscopic characteristics of blastomycosis are not identical with those of tuberculosis. In the tabu-

Duration and Outcome

The patients who came to autopsy had the disease for periods of between 3 and 24 months, so far as could be judged from their histories and physical evidence. Of the patients who had bone lesions, two died and two were living, 2 and 4 years after onset, without evidence of blastomycosis. One, a child of 5 years, who had osteomyelitis of a rib and miliary pulmonary involvement, revealed by x-ray examination, became free from evidence of the disease without iodides or other forms of therapy except drainage of the focus of osteomyelitis. Another patient was cured by a combination of therapeutic procedures.[14]

Of the two patients with resections of deep tissues, one is living and apparently free of blastomycosis 7 years after the onset of symptoms[3] (Martin and Smith,[1] case no. 10). The other patient with thoracic involvement and a supraclavicular subcutaneous abscess, cannot be traced.

Of the 13 patients with cutaneous involvement, two died 4 and 5 years after the onset, apparently of blastomycosis. Two showed no blastomycosis 2 and 5 years after onset. Most of the cutaneous cases have remained such without generalization, and have improved or have been cured by various forms of therapy. One has had a duration of 13 years, with involvement of the skin of the entire thorax and neck, but without impairment of general health.

Fundamental Gross Characteristics

The pyogenic character of the disease was usually impressive. Three of the patients coming to autopsy presented massive, fluctuating, subcutaneous abscesses (Text-Fig. 1) and all of them had osteomyelitis with sinuses extending to the skin or to joint surfaces. Brain, psoas and intramuscular (Figs. 3 and 4) abscesses were noted. The pus was usually pink. In a patient with a deep lesion, subjected to laparotomy, 25 cc. of creamy pus came from the tubes and pelvic peritoneal recesses.[3] In the cutaneous cases pus could be obtained from miliary abscesses at the periphery of the lesions.

Grossly the appearance of caseation was sometimes noted. In autopsy no. 2, the cut surface of the lung showed "large confluent areas of caseation from which pus can be squeezed." In autopsy no. 1, the fresh lungs at the time of autopsy showed "scattered, small yellow abscesses." After fixation, the same foci were spoken of as tubercles. In autopsy no. 3 the term "tubercles" was used for the pulmonary nodules, but it was noted that central softening was more apparent than in tuberculosis.

either severe systemic infection or with mild, localized cutaneous infection. When antibodies were high or when the patient was in the terminal anergic state, great numbers of organisms were present and caseous necrosis was often noted.

Discussion

In affirming the characteristic nature of the tissue response in blastomycosis, I am impelled to comment on the opposite point of view as expressed by Medlar.[8] He emphasized the identity of the gross and microscopic pathology in fungous and tuberculous infections in connection with a report of two cases of pulmonary blastomycosis. This statement seems to me to be far too sweeping even if the problem is approached as Medlar approached it, by comparison of the blastomycotic reaction with the histogenesis of tuberculosis, with special regard to the polymorphonuclear neutrophil, caseation and the presence of reticulum. In generalizing about blastomycosis, moreover, he used case reports of pulmonary blastomycosis alone upon which to base his opinion. It has been shown above that these are the very cases which may resemble tuberculosis most strongly.

Abscess, a circumscribed collection of pus, with the connotation of a liquid state in the unfixed condition at body temperature, may occur in tuberculosis, it is true. But if 23 cases of tuberculosis were accepted *seriatim,* the observer would certainly be far less impressed by the presence of abscesses grossly and microscopically than in the 23 cases of blastomycosis here reported.

The reverse of this would be true with respect to caseation. The term caseation is applied to the gross characteristics of the necrotic material commonly seen in tuberculous infection. If this term is applied to the necrotic material commonly seen in blastomycotic infection, in some instances the microscopic character of caseation in the two diseases will correspond closely. But in other instances, as has been pointed out, blastomycotic caseation may consist largely of masses of dead blastomycetes and not of reacting cells of the host. In tuberculosis this situation is encountered only in those very rare instances in which masses of stained tubercle bacilli can be perceived in a histologic section with the naked eye.

Miller,[17] using Bielschowsky's silver method (which is excellent to demonstrate blastomycetes and reticulum), showed that the growth and transformation of collagenous tissue differed in no way in the tubercle of blastomycosis from that in the tubercle of tuberculosis. While this is a point of resemblance between the reactions of the two diseases, it should be remembered that the production of reticulum

lation, similarity to tuberculosis was designated 1 plus (+) in seven cases and 2 plus (++) in one case. But even in these cases, other areas deviated from the characteristics of tuberculosis.

Iodides

An attempt was made to correlate the histologic picture and the number of living or dead organisms with the administration of iodides. No clear-cut conclusions could be adduced. In four of the cases massive doses had been given shortly before histologic examination. Two of these were cases examined at autopsy. Comparison with the other two similarly examined, in which iodides had not been given, showed no essential differences with respect to the organisms, the degree of caseation, or the fibrosis. In a surviving systemic case [3] in which laparotomy had been performed, desensitization and iodide therapy were carried out according to the method of Martin and Smith.[11] In the pelvic material there were blastomycotic tubercles with more fibrosis about them than in any other case (Fig. 7). In the same region, however, there were other areas with pus and organisms, and with all stages of inflammatory reaction and repair. (The reader is referred to the illustrations in the paper by Hamblen, Baker and Martin.[3]) In a cutaneous case, healing of lesions with dense fibrosis, devoid of blastomycetes, occurred. Iodides and desensitization had been used, but x-ray treatment had also been given. Thus the evidence as to the effect of iodides on the histologic picture of blastomycosis is not clear; possibly fibrosis is stimulated. Clinically, the administration of potassium iodides by mouth is said to promote marked improvement in the majority of cutaneous cases, but in the systemic cases the results are very discouraging.[11]

It has not been possible to evaluate histologically the effect of x-ray treatment in this series, since there were not enough biopsies after such treatment. Clinically the cutaneous lesions appear to undergo regression and fibrosis with x-ray treatment,[16] but usually too many factors have been present in the therapy to evaluate any one of them properly.

Immunologic and Allergic Responses

The pathologic analysis was made with the following observations of Martin and Smith [1] in mind:

"Antibodies can be found in the sera of patients who are heavily infected; they persist until death unless the infectious process is overcome or greatly reduced.

"Some patients develop a condition of hypersensitiveness to the infecting fungus, and this allergic state diminishes in the terminal stages of the disease."

A correlation of histopathologic details with such clinical observations could be made only in so far as these details were associated with

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and collagen is a general pathologic process related to a stage in repair, and not peculiarly associated with these diseases, nor with all stages of these diseases.

Summary and Conclusions

1. Among 23 cases of human blastomycosis (13 cutaneous and 10 generalized or thoracic) complete autopsy was available in 4, and histologic material in the remaining.

2. The formation of abscesses was an impressive gross feature of the generalized cases, and polymorphonuclear foci were noted microscopically in all 23 cases. Giant cells were always present. Caseation was present in the generalized and thoracic cases, but was not noted in the cutaneous cases.

3. Human blastomycosis was interpreted as being primarily pyogenic, with prominence of polymorphonuclear neutrophils. Some lesions of some cases, especially in the systemic group, closely resembled the lesions of tuberculosis.

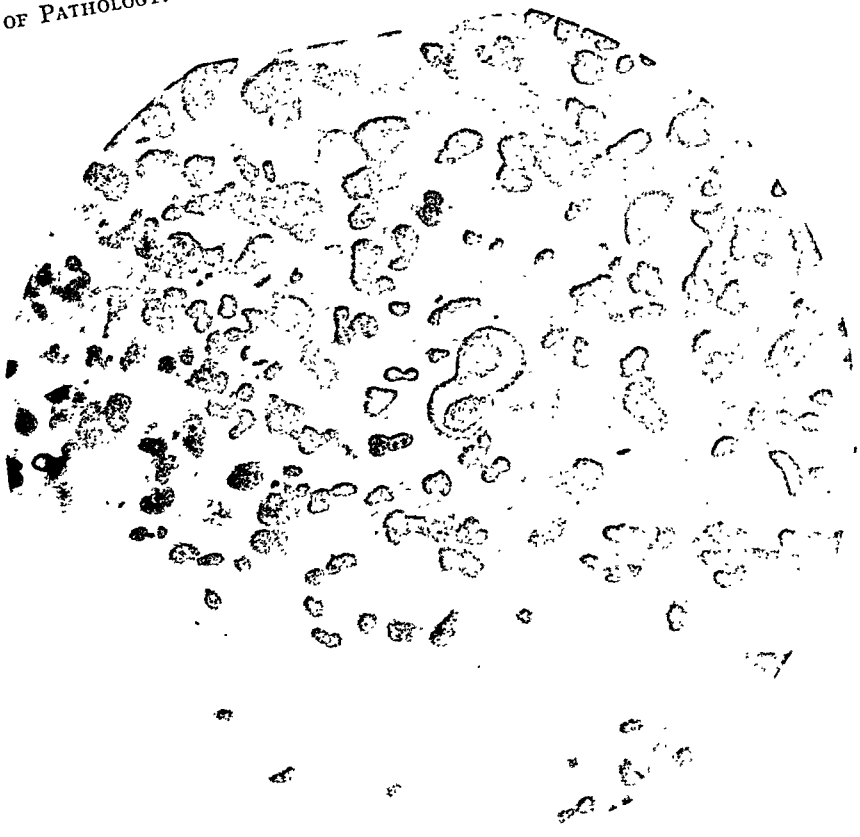
4. The terminal stage of systemic blastomycosis in man corresponded closely to the experimental disease in the mouse. Masses of blastomycetes occurred with necrosis, producing a toxic effect upon the patient. In the cutaneous cases, in contrast, organisms were usually moderate in numbers, caseous necrosis was usually absent and the lesion was composed of miliary abscesses, granulation tissue and hyperplastic epidermis. There was little toxic effect upon the patients.

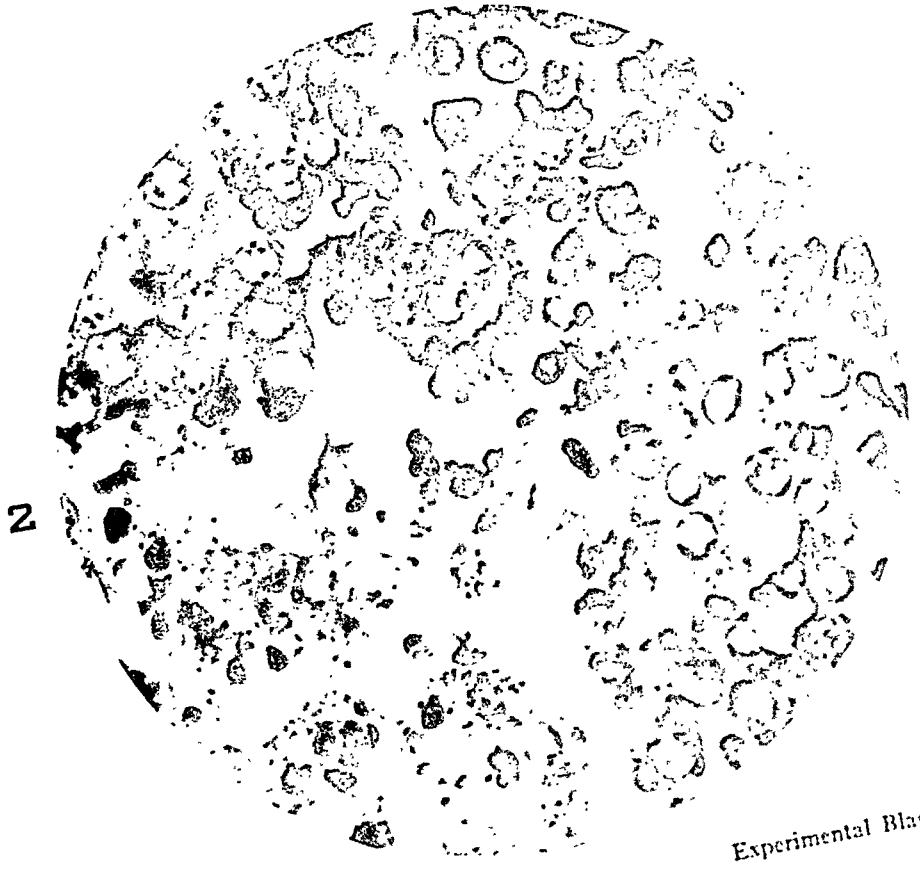
5. These observations on the nature of toxicity in blastomycosis suggest that therapeutically a fungicide is not desirable in the severe systemic cases, since too much necrotic blastomycotic material is already present.

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2

Experimental Blastomycosis in Mice

DESCRIPTION OF PLATES

PLATE 78

FIG. 1. Microscopic abscess in cutaneous blastomycosis. Blastomycetes, one budding, are free in the center. There is a predominantly polymorphonuclear response, but other types of cells are present. The epidermis appears below. From skin of nose of white man, 63 years old, with multiple cutaneous lesions. Biopsy was taken 3 years after onset of disease. The patient was living in 1938, 2 years later, without evidence of blastomycosis. × 705.

FIG. 2. Massive growth of blastomycetes in an abscess of the human brain, autopsy no. 2. (Photograph of the gross specimen is shown in Fig. 9.) Viable blastomycetes, from the edge of the abscess, are above; and necrosis, toward the center of the abscess, is below and to the left. × 705.

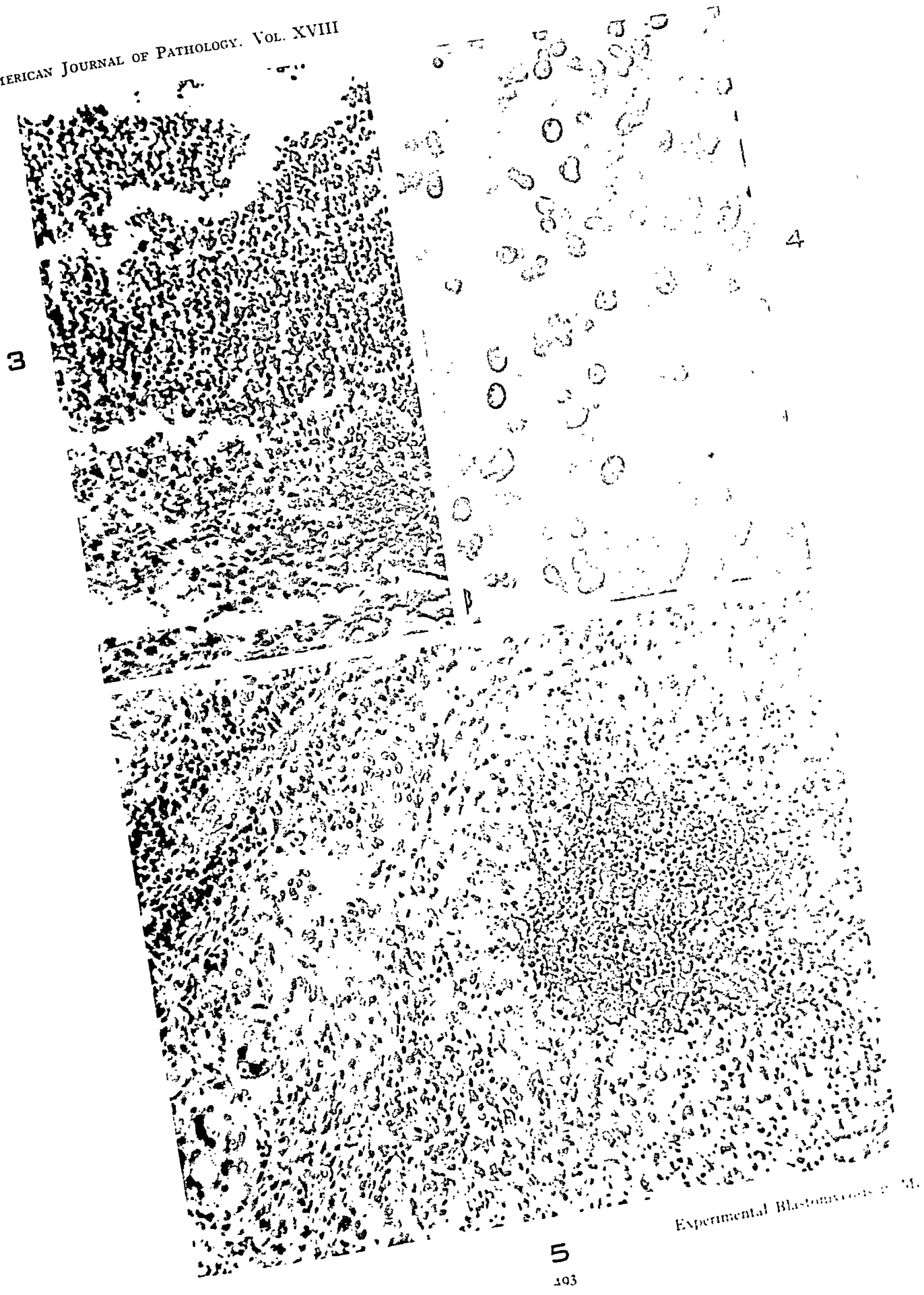

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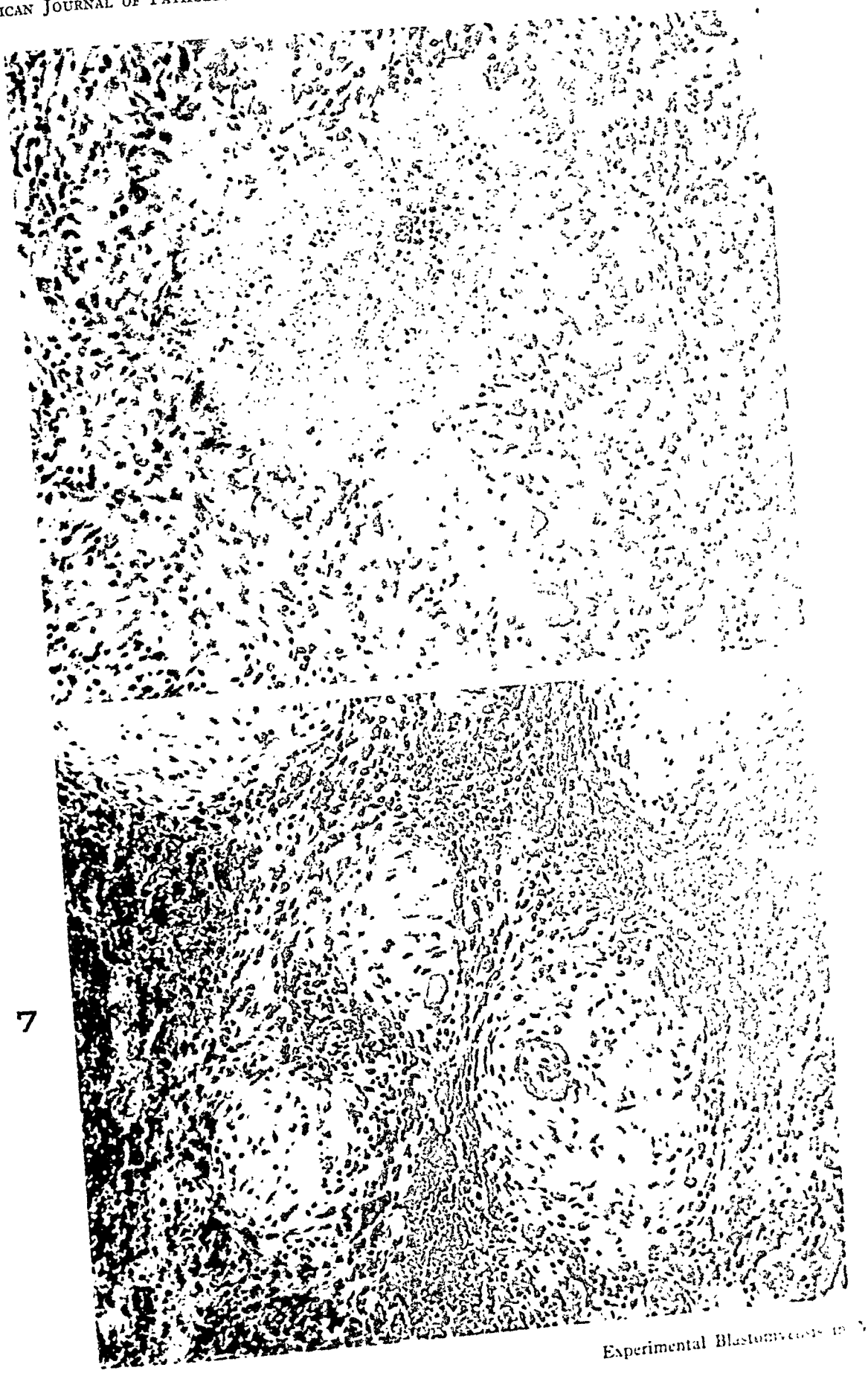
PLATE 79

FIG. 3. Wall of an abscess, and exudate, in muscle of upper arm, autopsy no. 2. × 174.

FIG. 4. Higher power magnification of area in Figure 3 to show free blastomycetes with cellular response rich in polymorphonuclear cells. Mononuclear cells are also numerous. × 760.

FIG. 5. Abscess and giant cells containing blastomycetes in an osteomyelitic case. This was a surgical specimen. Osteomyelitis of elbow and rib in a white male, 30 years old. The patient was apparently cured by a variety of therapeutic methods and was well 2 years after onset. (See report of Martin and Jones.[14]) × 174.

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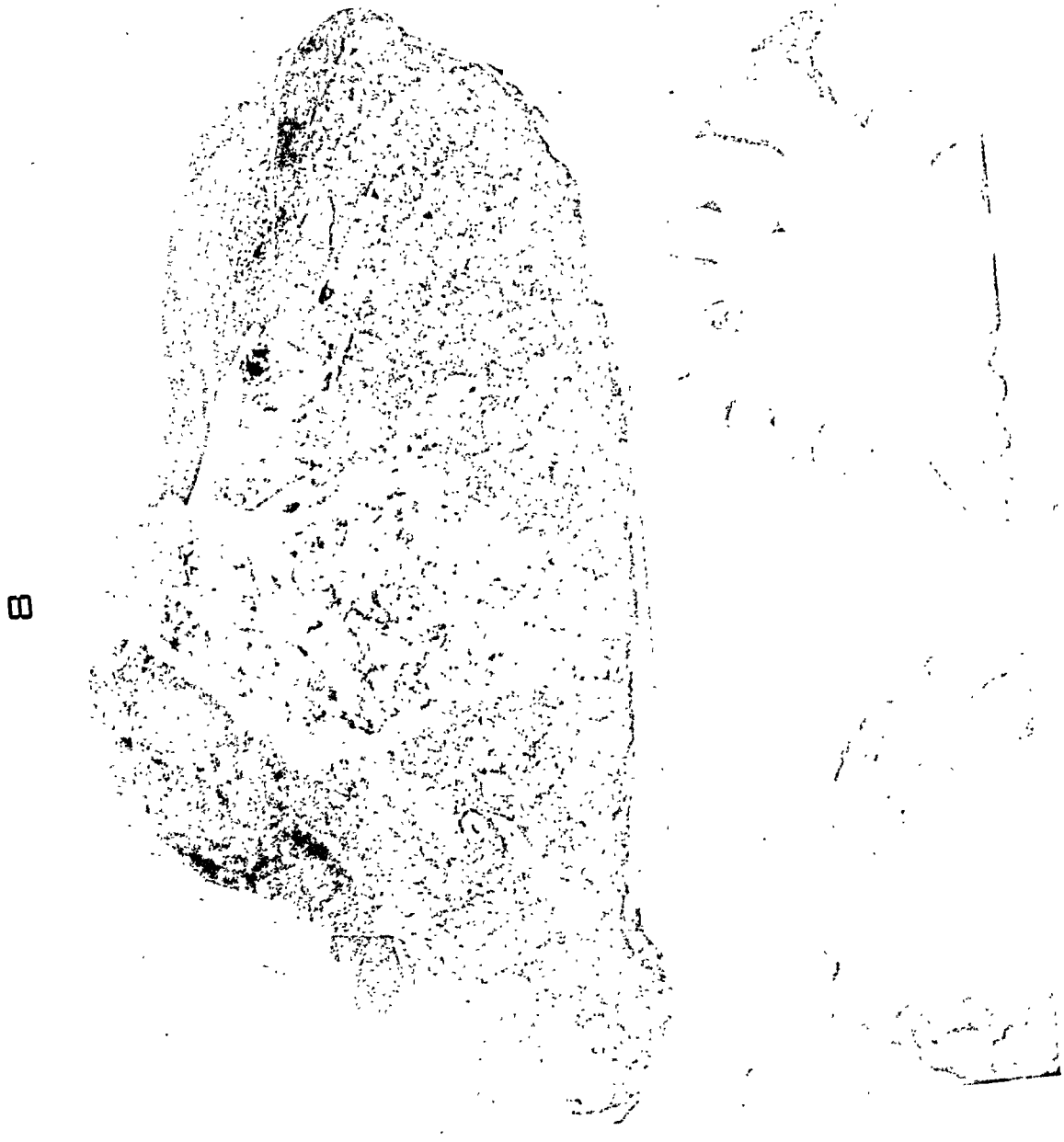
Experimental Blastomycosis in X

PLATE 80

FIG. 6. Caseous pulmonary lesion in autopsy no. 3, upon a case of generalized blastomycosis. Shadows of necrotic blastomycetes were seen in several levels of this lesion and in the adjacent giant cells. Acid-fast bacilli were not found in sections appropriately stained. × 174.

FIG. 7. Hard tubercles in peritoneal reaction about blastomycosis of fallopian tubes. Surgical specimen from white woman, 27 years of age, with generalized blastomycosis, now (1941) apparently free of the disease, 7 years after onset.[3] Blastomycetes were present in adjacent tubercles. Acid-fast bacilli were not found. × 174.

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B

Experimental Blastomycosis in Mice

Baker

PLATE 81

FIG. 8. Blastomycotic pulmonary abscess (autopsy no. 2).

FIG. 9. Blastomycotic brain abscesses (autopsy no. 2).

PATHOLOGY AND PATHOLOGIC DIAGNOSIS OF RADIATION LESIONS IN THE GASTRO-INTESTINAL TRACT *

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The occurrence of lesions in the stomach and intestine as a consequence of radiation therapy is well known. Their nature, frequency and pathogenesis are dealt with in a current review [1] and in other publications,[2] so that material will not be repeated here. The purpose of the present paper is to review the radiation reactions of the gastro-intestinal tract studied in this laboratory and to present the pathologic findings in detail. It is hoped to emphasize those features which may be useful as diagnostic criteria of radiation reaction. With the increasing use of radiation therapy in high doses, lesions are being frequently encountered with which pathologists should be familiar. A determination on pathologic grounds whether a given lesion is entirely or in part due to radiation may have importance for further therapy.

The 38 cases employed for study were selected from a considerably larger group in our files. The cases selected were those in which the lesions showed a marked radiation reaction and in which the details of radiotherapy were available. An outline summary of these cases is presented (Table I).

The lesions may be grouped (Table II) according to their location in the gastro-intestinal tract and the nature of the reaction. They include ulcers, fistulae and strictures. A reaction comprising fibrosis and scarring, with thickening and induration of the bowel wall in the absence of ulceration, has been termed "sclerosis." Sclerosis alone may be marked enough to cause a stricture. Many reactions combined ulceration and stricture formation.

Many of the lesions developed at sites distant from neoplastic tissue but some occurred at the site of a partially or completely destroyed tumor and must be considered as "mixed" lesions. For example, in the bases of some ulcers exhibiting radiation reaction there was widespread infiltration of neoplastic tissue. However, since the radiation in most cases had been directed at a malignant process, many of the radiation lesions were in proximity to neoplastic tissue although they themselves were free of tumor. For example, in one case rectal ulceration characteristic of radiation reaction was present, while the

* Received for publication, August 14, 1941.

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7. Carcinoma of cervix		4,900		7 years	Resection	Ileum (adherent to pelvic brim)	Multiple (2) ulcers with – stenosis	None	None
8. Carcinoma of cervix	6,000	4,000		8 months	Resection	Ileum (adherent to pelvic brim)	Multiple (5) ulcers with stenosis	None	None
9. Carcinoma of cervix	13,000	3,500	17 months	7 months	Resection	Small intestine (free loop)	Ulcer with stenosis	None	None
10. Carcinoma of retained cervical stump		3,500		3 months	Resection	Small intestine (adherent to cervical stump)	Entero-cervical fistula	None	None
11. Carcinoma of cervix	7,600	3,400	3 months	2 months	Necropsy	Ileum (adherent to uterus)	Ileo-uterine fistula	None	None
12. Carcinoma of rectum	1,500	7,800		3 months	Necropsy	Jejunum (adherent to rectum)	Ulcer	None	None
13. Generalized Hodgkin's disease	1,900		4 years	1 month	Necropsy	Small intestine	Multiple ulcers with perforation	None	Probable regressed Hodgkin's tissue at site
	and additional treatment dose unknown								
14. Ewing's tumor of ilium	3,500		4 months	Immediate	Necropsy	Appendix	Sclerosis	None	None
15. Carcinoma of uterus		6,700	2¼ years	4 months	Necropsy	Sigmoid	Sclerosis	None	None
16. Carcinoma of rectum		2,500	2 months	5 months	Resection	Rectum	Ulcer with stenosis	None	None
17. Carcinoma of cervix		5,500		11 months	Necropsy	Rectum	Sclerosis	None	None
18. Carcinoma of anus, recurrent following resection		16,500	5 months	6 months	Necropsy	Sigmoid	Sigmoido-uterine fistula	None	None
19. Carcinoma of cervix		7,000	4 years	20 months	Necropsy	Rectum	Recto-uterine fistula	None	None
20. Carcinoma of cervix	3,500	5,200	7 months	1 month	Necropsy	Rectum	Ulcer	None	None
				7 months	Resection	Sigmoid	Ulcer	None	None
21. Carcinoma of anus		900		5 months	Necropsy	Rectum	Ulcer	None	None
22. Carcinoma of cervix	6,400	3,000	3 months	4 months	Resection				
23. Carcinoma of rectum	2,900	4,500	3 months						

TABLE I

Summary of 38 Cases Showing Radiation Lesions of the Gastro-Intestinal Tract

Primary disease	Radiotherapy		Duration of treatment (if multiple courses)	Interval from completion of treatment to time of pathologic examination	Method by which tissue was obtained	Radiation lesion		Neoplastic infiltration as part of lesion
	Roentgens	Milligram or millicurie hours				Site	Nature	
1. Tumor of stomach	5,400			1 week	Resection	Stomach	Ulcer with chronic perforation	Nodules of necrotic and unidentifiable tumor in base of ulcer; leiomyoma (?)
2. Generalized giant-follicular lymphoma	3,000		17 months	2 months	Necropsy	Stomach	Ulcer	Probable regressed lymphoma at site
3. Carcinoma of cervix recurrent following panhysterectomy	7,200			7 months	Necropsy	Ileum (adherent in pelvis)	Ileo-vaginal fistula	Present
4. Carcinoma of rectum	8,900		26 months	3 months	Necropsy	Small intestine (adherent to retroperitoneal metastases)	Ulcer	Present
5. Carcinoma of retained cervical stump		3,500		1 month	Resection	Ileum (adherent to cervical stump)	Sclerosis with stenosis	None
6. Carcinoma of cervix		4,700		7 years	Resection	Ileum (fresh adhesions to broad ligament; laparotomy 1 year earlier for gastro-intestinal symptoms and not adherent then)	Multiple (2) ulcers with stenosis	None

perirectal tissues were extensively invaded by carcinoma that arose in the cervix. In another case carcinoma of the rectum had undergone extensive necrosis following radiotherapy, while the nearby sigmoid showed typical radiation sclerosis. It is felt that such lesions should be considered as radiation reactions independent of the neoplastic process.

TABLE II

Distribution of the Lesions in the Gastro-Intestinal Tract and Their Relationship to the Presence of Neoplasm

	Radiation reaction without tumor			Radiation reaction with tumor		
	Ulcer or fistula	Ulcer with stenosis	Sclerosis or stenosis	Ulcer or fistula	Ulcer with stenosis	Sclerosis or stenosis
Large intestine	10	1	6	5	1	1
Small intestine	3	4	1	3	..	..
Stomach	..	..	..	2	..	..
Appendix	..	..	1	..	..	..

PATHOLOGIC ANATOMY

The lesions consisted of ulceration, sclerosis and combinations of the two. The process sometimes involved a segment of intestine uniformly; in other instances it was focal, with single or multiple lesions.

The bowel wall was usually thickened and indurated, with the serosa opaque and showing prominent telangiectasia. The mesentery, particularly at its point of attachment, was frequently similarly involved. The mucosa rarely appeared entirely normal, but usually, even in non-ulcerated lesions, atrophy and fixation to the submucosa were seen. The degree of ulceration ranged from confluent, irregular, superficial erosions to deep, punched-out ulcers. The persisting mucosa was irregularly heaped up and nodular in the former, while discrete ulcers were very sharp-edged. Telangiectasia occurred especially at the edges of ulcerated areas. Stenosis was sometimes due to diffuse sclerosis with general constriction of a segment, and sometimes to formation of a stricture at a site of ulceration.

Secondary changes, such as inflammatory reactions related to penetrating or perforating ulcers and fistulae, were evident. Exudation of fibrin, fibrinopurulent membranes and adhesions were encountered. Necrosis was a part of most reactions and in extreme instances the reaction approached massive gangrene of a loop of intestine.

TABLE I—*Continued*

Primary disease	Radiotherapy		Duration of treatment (if multiple courses)	Interval from completion of treatment to time of pathologic examination	Method by which tissue was obtained	Radiation lesion		Neoplastic infiltration as part of lesion
	Roentgens	Milligram or millicurie hours				Site	Nature	
24. Carcinoma of vagina		8,600		4 months	'Resection	Rectum	Recto-vaginal fistula	None
25. Carcinoma of rectum		1,600		9 months	Resection	Rectum	Sclerosis	None
26. Carcinoma of rectum recurrent following local resection		3,000	5 months	8 months	Resection	Rectum	Ulcer	None
27. Carcinoma of rectum	6,000	3,800	8 months	8 months	Resection	Rectum	Ulcer	None
28. Carcinoma of cervix	7,200	2,800		5 months	Biopsy	Rectum	Ulcer with stenosis	None
29. Carcinoma of cervix		2,800		3 months	Necropsy	Rectum	Ulcer	None
30. Carcinoma of rectum	10,000		5 months	9 months	Necropsy	Rectum	Sclerosis	None
31. Carcinoma of cervix	4,800	8,100	4¾ years	2 months	Necropsy	Rectum	Recto-cervical fistula	None
32. Carcinoma of cervix	4,000	3,000	2 months	10 months	Necropsy	Rectum	Sclerosis	None
33. Carcinoma of cervix	1,800	6,900	6 months	2 months	Necropsy	Rectum	Recto-vaginal fistula	Present
34. Carcinoma of rectum	2,800	3,600	2 months	2 weeks	Necropsy	Rectum	Ulcer	Present
35. Carcinoma of rectum		2,600	14 months	6 months	Resection	Rectum	Ulcer	Present
37. Carcinoma of rectum	6,300			2 months	Necropsy	Rectum	Ulcer	Present
36. Carcinoma of rectum		1,700	4 months	9 months	Resection	Rectum	Ulcer	Present
38. Carcinoma of rectum	8,400			1 month	Resection	Rectum	Sclerosis	Present

were thromboses. The vascular changes were present in nonulcerated lesions, but with ulceration the local inflammatory process was accompanied by more marked fibrinoid necrosis, thrombosis and vascular sclerosis.

Mucosa

In ulcerative processes the epithelial lining was destroyed. There were, however, regenerative and healing attempts which differed little from those seen in other types of ulceration. Flat or cuboidal undifferentiated epithelium grew in from the ulcer edge, while remnants of glands within the ulcerated area sometimes became hyperplastic and in them cells of the mucous type were extremely prominent.

Even when the mucosa was not ulcerated it underwent profound alteration and sometimes atrophy. Mucous stasis with overproduction was a very common finding and was far more striking than is commonly seen in other conditions. The goblet cells were large and full and were present in great numbers. The glands showed various forms of distortion to varying degrees. They became short and wide-mouthed or, conversely, sometimes became very narrow and elongated, lined by flattened epithelium, or were dilated and cystic. Among the cells were frequently seen bizarre and atypical forms, even multinucleate and giant-cell elements.

In many cells the swelling of the nucleus and the prominent nucleolus and chromatin with an "owl's eye" appearance were characteristic. Varying types of cellular degeneration were seen, ranging from hydrops to necrosis. In one specimen an unusual change was noted—a marked degree of vacuolation of the parietal cells of the stomach.

Muscle

In many instances the muscle coats were also altered. Edema and vacuolation of the fibers were sometimes present, and in instances of marked change, degeneration and atrophy of fibers were widespread. Interstitial fibrosis with hyalinized connective tissue accompanied these changes, and hyaline degeneration and swelling of muscle fibers resembling Zenker's degeneration were sometimes seen. The combination of atrophic fibers, swollen fibers and hyaline interstitial fibrosis was very striking. In one case there were large, bizarre, atypical muscle cells in the muscularis mucosae. The abnormal muscle cells in vessel walls have been previously described.

Necrosis and Inflammation

Necrosis was extensive in the ulcers. While the necrotic tissue did not always present specific features, it was sometimes characteristic in

Histopathology

Connective Tissue

The changes in the connective tissues involved the mucosa, submucosa, vessel walls, stroma of the muscle layers and the serosal or adventitial tissues. There was usually edema of some degree and in early reactions edema was sometimes the dominant stromal change. There was frequently precipitated protein as well as an exudate of fibrin, with little cellular reaction to it as it lay unchanged and unabsorbed. In more advanced stages of reaction the characteristic "hyaline" change of the collagen, in which the collagen bundles are swollen, glassy and afibrillar, was seen. The hyaline change suggests "setting" or "gelling" of the edematous connective tissue and may superficially resemble amyloid. Special staining of the hyaline connective tissue revealed that there was swelling of the ground substance but usually relatively little increase in collagen fibers, reticulin fibers, or elastic fibers. The extent of actual fibrosis in the sense of new formation of fibrous tissue accounted only in part for the increased tissue mass of the hyaline connective tissue.

The hyaline thickening and edema of the connective tissue increased the thickness of the bowel wall considerably, especially in the submucosal and subserosal layers. The attached mesenteric tissues were similarly involved. In one instance the basement membrane of the surface epithelium was hyalinized and thickened.

The fibroblasts exhibited an atypical structure reminiscent of neoplastic change. Their processes were large and distorted. The nuclei were swollen, giant, bizarrely shaped, lobulated and even multiple, with prominent nucleoli and chromatin masses standing out darkly against a pale background in "owl's eye" manner.

Vessels

In the arteries, edema of the wall was seen early; and later, hyaline change quite comparable to that seen in the connective tissue elsewhere was noted. The muscle cells were sometimes hypertrophied and distorted. Sclerosis was far less marked in the arteries than it was in the veins. Obliterative endophlebitis was sometimes extreme with occasionally some splitting and reduplication of the elastica about the periphery. Lymphatic and venous ectasia together provided the most striking vascular feature of the reaction.

The endothelial cells in general rarely showed swelling and atypical cellular forms similar to those described for the fibroblasts. Deposits of fibrin in the vessel walls were sometimes seen, and occasionally there

forms, too, are rarely seen in other than radiation reactions. *Telangiectasia* involving the veins and lymphatics to a marked degree is very characteristic. It may even be seen in the gross specimen or in the lesion itself prior to removal. The *hyaline degeneration of vessel walls* requires no further comment, although the frequent presence of ordinary arteriosclerosis in the tissues of individuals from the higher age groups may be confusing.

The *secondary diagnostic features* include the endothelial abnormalities, phlebosclerosis, the changes in the muscle fibers and the epithelial alterations. Atypical endothelial cells are seen far less frequently than the almost invariably present altered fibroblasts, but when they are seen, they lend support to the diagnosis. Phlebosclerosis is an important part of the vascular change, but can seldom be seen clearly in a small specimen. It is also seen, of course, in many other types of inflammatory reaction as a residue of phlebitis and consequently is not a specific finding. Stains for elastica may bring out details that would otherwise be missed and many obliterated and partially obliterated veins were overlooked in our preparations until this method was used. The degeneration of the muscle coat may be evident in sections from a complete specimen but is seldom of value in a biopsy fragment.

Finally, the epithelial changes described are not necessarily characteristic of radiation reaction. Atypical glands, hyperplasia, regenerating epithelium and even the mucous change may be seen in any process that has damaged the mucous membrane. However, swollen nuclei with prominent "owl's eye" nucleoli, and marked mucous change are far more frequent in radiation reactions.

NOTE: We are indebted for clinical data to the Surgical Staff of the Palmer Memorial Hospital, particularly Dr. George A. Leland, Jr.; and to the Staff of the Lahey Clinic, as well as to Dr. Joseph H. Marks, radiologist to the New England Deaconess Hospital, and to Dr. Max Ritvo and Dr. F. W. O'Brien.

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1. Friedman, N. B. The stomach and intestine. In: Warren, Shields. The effect of radiation on normal tissues. *Arch. Path.* (In press.)
2. Friedman, N. B., and Warren, Shields. Evolution of experimental radiation ulcers of the intestine. *Arch. Path.*, 1942, 33, 326–333.

showing a distinct, glassy, homogeneous, acidophilic structure similar to that produced by a physical agent such as the heat of a cautery.

The inflammatory reactions were quite varied and all types of cellular response were seen. Granulation tissue frequently formed in the usual manner although occasionally the degenerated hyalinized tissues remained almost avascular.

Diagnosis

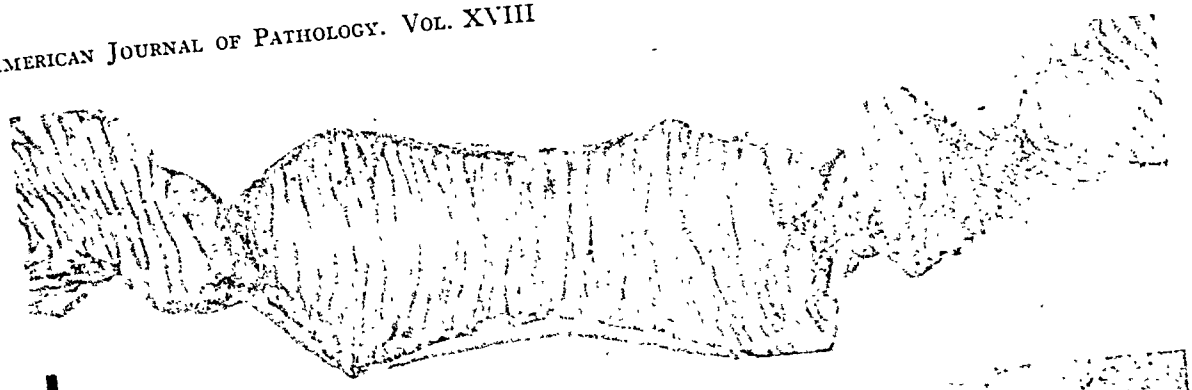
Knowledge of the changes seen in radiation reactions is of value from two points of view. On the one hand these changes are of academic and biologic interest in considering lesions studied at necropsy or in surgical material. The descriptions given in this paper give a perspective of the tissue changes seen in the gastro-intestinal tract in a moderately broad selection of material. It is evident that further study of the mucosal changes and of the gastric mucosa in particular is needed.

The second and more immediately practical point of view is that of the value of these criteria in making diagnoses on biopsy material. The differential diagnosis on pathologic grounds between the extension of a malignant process and a radiation reaction may be important. A common example is the problem of an ulcerated rectum in a patient irradiated for carcinoma of the cervix. A pathologic diagnosis of "radiation reaction" rather than "chronic inflammation," if it can be made, may be invaluable in deciding what therapy should be instituted. Therefore a brief review and summary of those changes which are likely to be useful in diagnosis will be presented.

The significant changes may be divided into primary and secondary criteria, that is, those without which the diagnosis cannot be made and those which are merely supportive. It is, of course, obvious that a biopsy specimen submitted for diagnosis may represent only a small part of the lesion. The primary points to be looked for are hyalinization of the connective tissue, abnormal fibroblasts, telangiectasia and hyaline degeneration of vessel walls. These have all been described in detail and illustrated.

Hyalinization of radiation reaction may be simulated by the hyaline fibrous tissue of chronic inflammation, but with experience the peculiar swollen, glassy, afibrillar matrix of radiation reactions can be fairly well distinguished. Rapidly proliferating *fibroblasts* in organizing and healing processes and in active granulation tissue somewhat resemble those seen in radiation lesions. However, in radiation reactions they are less abundant, show little mitotic activity and tend to be stellate and large rather than of the narrow spindle-shaped variety. Abnormal nuclear

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Gastro-Intestinal Radiation Lesions

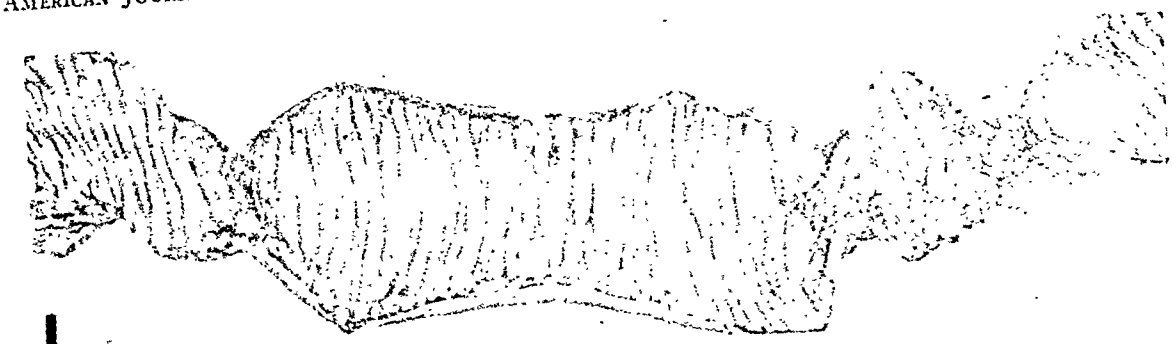
DESCRIPTION OF PLATES

PLATE 82

FIG. 1. Case no. 8. Multiple radiation ulcers and strictures of small intestine.

FIG. 2. Case no. 9. Regenerating epithelium at edge of radiation ulcer of small intestine. × 125.

FIG. 3. Case no. 1. Hyperplastic glands and mucous change in radiation ulcer of stomach. × 125.



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Gastro-Intestinal Radiation Lesions

Warren and Friedman

DESCRIPTION OF PLATES

PLATE 82

FIG. 1. Case no. 8. Multiple radiation ulcers and strictures of small intestine.

FIG. 2. Case no. 9. Regenerating epithelium at edge of radiation ulcer of small intestine. × 125.

FIG. 3. Case no. 1. Hyperplastic glands and mucous change in radiation ulcer of stomach. × 125.

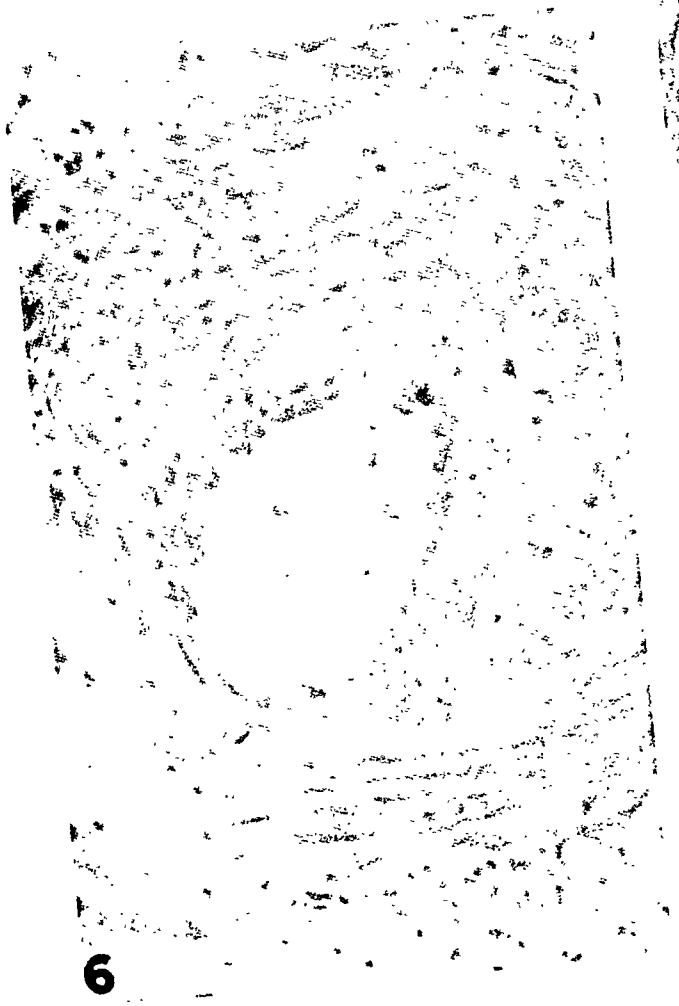
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Gastro-Intestinal Radiation Lesions

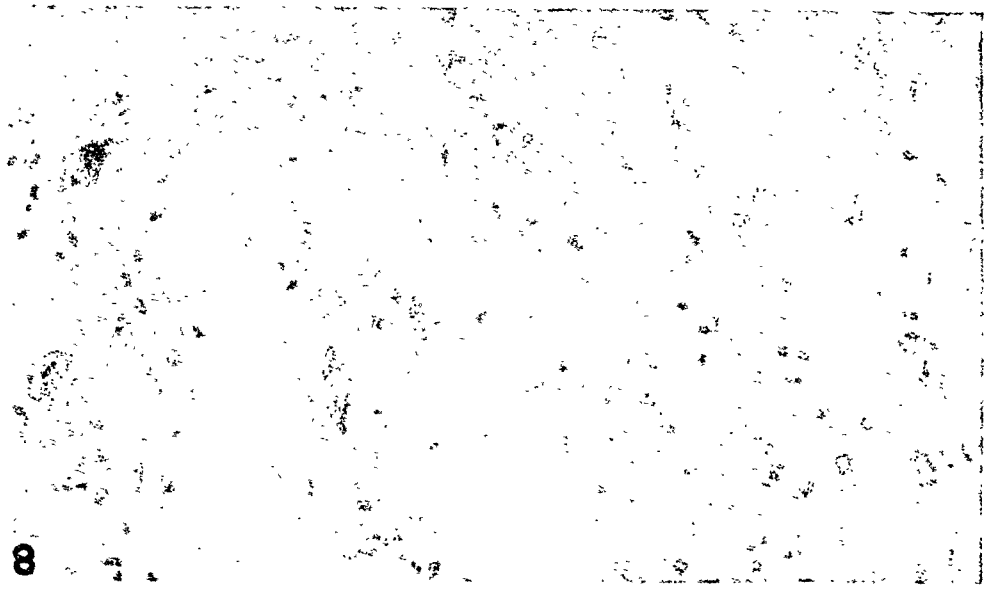
PLATE 83

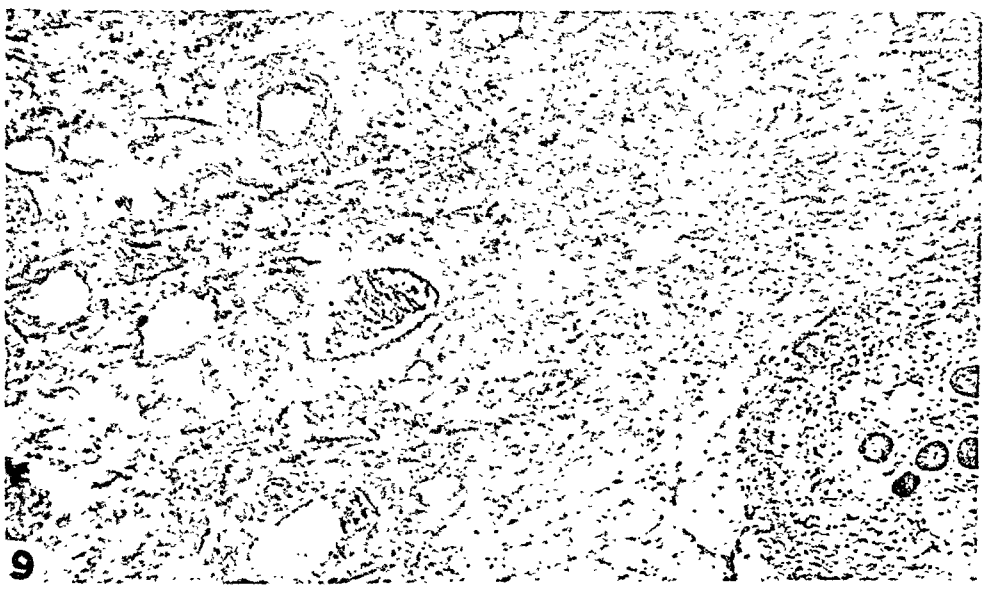
FIG. 4. Case no. 1. Vacuolation of parietal cells in radiation reaction of gastric mucosa. × 125.

FIG. 5. Case no. 1. Same as Figure 4. × 500.

FIG. 6. Case no. 23. Hyalinized artery in radiation ulcer of rectum. × 200.

FIG. 7. Case no. 8. Phlebosclerosis in radiation reaction of small intestine. Elastica stain. An artery without significant change is seen. × 100.


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PLATE 84

FIG. 8. Case no. 29. Abnormal fibroblasts and hyalinized collagen in radiation ulcer of rectum. × 265.

FIG. 9. Case no. 9. Edema and telangiectasia of submucosa in radiation reaction of small intestine. × 55.

FIG. 10. Case no. 13. Radiation reaction in muscularis of small intestine. × 170.

THE LESIONS PRODUCED IN THE GASTRO-INTESTINAL TRACT BY IRRADIATION *

General Review with an Illustrative Case Report

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The pathologic anatomy of the injury to the gastro-intestinal tract caused by irradiation has been described by a number of investigators. It is the object of this paper to summarize the human cases and the experimental studies from the literature and to add a case of roentgen-ray intoxication of unusual interest. The data included cover only those instances where roentgen rays alone, or in combination with some radioactive substance, have been employed in the treatment of various lesions. Total dosages are stated when available, but the reader is referred to the original articles for other technical details.

Human Cases

Rolleston [1] reviewed the harmful effects of irradiation by both x-rays and radium and listed the symptoms noted after deep irradiation. The severe constitutional symptoms are nausea, uncontrollable vomiting (sometimes hematemesis), offensive diarrhea, melena, abdominal pain and distension, fever up to 104° F., restlessness, profound prostration, progressive cardiac failure, small and rapid pulse and dyspnea.

The first cases with clinical evidence of injury to the gastro-intestinal tract by irradiation were reported by Walsh [2] in 1897. Although deaths were recorded [3] following irradiation therapy, it was not until 1917 that a fatal case with autopsy studies was presented by Franz and Orth.[4] A woman, 35 years old, who had inoperable carcinoma of the cervix, received 2800 units of x-rays in three courses over a period of 5 months. Between the second and third series her weight declined from 112 to 95 pounds. Severe, profuse diarrhea developed after the last series, culminating in death at the end of the fifth month. Necropsy revealed tanning of the abdominal skin, carcinomatous infiltration of the uterus and cervical canal, severe enteritis of the small intestine and ulceration of the large intestine. Microscopic examination revealed heavy cellular infiltrations and hemorrhages in the intestinal ulcerations, masses of bacteria and necrotic tissue, general submucosal edema, cells giving a strong fat reaction with Sudan stain, hyalinization of the mucous membrane, edema and hemorrhage in the muscularis and thickening of the walls and stenosis of the veins.

Von Franqué [5] treated a woman having endometrial hyperplasia with three courses of x-rays. Two weeks after the last series, radiation reaction of the abdominal skin, vomiting, tympanites, profuse, watery diarrhea and severe colic began and subsided slowly. Heck,[6] 16 months later, found the patient to have an intestinal-cutaneous fistula. At operation, friable muscle, thickened peritoneum and deep fascia, extension of the fistula into the cecum and scarring of the ileocecal valve were disclosed. The distal 20 cm. of ileum was resected and the stump of ileum was anastomosed to the side of the ascending colon. After operation the edges of the wound became gangrenous and the sutures cut through, allowing the wound

* Received for publication, August 14, 1941.

Mühlmann and Meyer[10] reported the case of a woman, 56 years old, with cervical carcinoma who had 3450 mg. hours of radium bromide and an unstated amount of x-ray irradiation over a 4 months' period. Seven and 10 months later she was given unstated doses of x-rays to the parametria. At the end of treatment she became bedridden and had melena and anemia with a hemoglobin of 24 per cent. Increased melena and abdominal pain preceded death. Findings at autopsy were: marked anemia; splenic atrophy; great distension of the sigmoid colon which was indurated, adherent to the pelvic wall and to the atrophic uterus, and was involved by a ring of necrosis and perforation 28 cm. proximal to the anus, communicating with a fecal abscess beneath the inguinal ligament, and phlegmon of the wall of the cecum. Microscopically the necrotic parts of the gut were limited by striped tissue overlaid on its inner surface by vessels with marked hyaline thickening or obliteration.

A woman, 42 years old, with hypermenorrhea and marked anemia was studied by Schwarz.[11] She received doses of 100 per cent H.E.D., 122 min., and 90 per cent H.E.D., 131 min., over the anterior and posterior abdominal areas for a period of 4 days. Supportive measures failed to control the ensuing diarrhea, melena and tenesmus. Four months later an abdominal mass of the left lower quadrant was felt. Operation revealed 12 cm. of the sigmoid colon to be white, hard and bound to the thickened left adnexa. A portion of the colon measuring 15 cm. was resected and an end-to-end anastomosis was performed. The resected piece of colon contained several ulcers extending to the serosa and rimmed by necrosis. Necrosis of the mucous membrane at the site of the ulcers breaking through into the submucosa and inner muscle coat, infiltration of inflammatory cells and fibrosis of the submucosa were present on microscopic examination.

A woman, 63 years of age, reported by Fried,[12] had total excision of the uterus for carcinoma followed by deep x-ray irradiation to a sacral and two anterior fields, 110 per cent H.E.D., 50 min., and 100 per cent H.E.D., 118 min. Four weeks later she had the typical appearance of peritonitis, diarrhea, tenesmus and tanning of the abdominal skin. She lost strength rapidly and died. Abdominal autopsy disclosed the following: pneumoperitoneum; intestinal distension; fibrous peritonitis; purulent peritonitis with fecal contamination; rupture of the sacral sigmoid colon; an indurated, stenosed rectum, and mucosal ulceration of the ileocecal valve. Histologically the rectal wall showed marked inflammation of the serosa, edema, foci of chronic inflammatory cells and swelling of the capillary endothelium.

Sanders[13] presented the case of a woman, 39 years old, with general weakness and profuse bleeding who received an unspecified amount of deep x-ray irradiation. Eight days later pigmentation of the skin, diarrhea and chronic ileus were evident. Six months later an operation disclosed a pale, contracted, friable loop of lower ileum adherent to the sigmoid colon and to the linea innominata of the pelvis. An end-to-side anastomosis was done. The resected ileal loop was 45 cm. long, shrunken to half its original volume, firm, thickened and stenosed in two places. One stenotic area was friable and studded with numerous hemorrhages; the other, 10 cm. long, was adherent to the cecum, bean-shaped, taut and dark red. Histologic examination revealed endarteritis and endophlebitis obliterans, nuclear destruction in the glandular epithelium, chronic inflammation and fibrosis in the submucosa and proliferation of vessels in the submucosa and muscularis mucosae.

Ball[14] presented the case of a woman, 41 years of age, with cervical carcinoma who made a good recovery following panhysterectomy. This was followed by a total of 150 e.s.u. of x-rays. The patient developed diarrhea, tenesmus, emaciation, tanning of the abdominal skin, melena and coma and died 38 days later. At autopsy there were observed: a dark chocolate abdominal musculature; adhesions at the site of the operative scar; no evidence of metastases; narrowing and thickening

to gape. Distension, ileus, intestinal obstruction and peritonitis developed and the patient died 11 days after operation. Autopsy revealed a fibrinopurulent peritonitis and a tight ileocecal anastomosis. The ileum resected at operation was rigid, thickened and edematous. Also observed were widening of the lumina of the glands, atrophy of the lymph follicles, covering of the mucosa by a simple layer of epithelium, absence of the crypts of Lieberkühn, ulceration of the mucosa, disintegration of the muscle coats, foci of lymphocytes in the intestinal wall and thickening and fragmentation of the intima and widening of the media of the blood vessels.

In an analysis of 127 cases treated for various conditions by x-rays, radium, or mesothorium, or by combinations of them, Haendly [7] demonstrated that irradiation can lead to the formation of ulcers of the intestinal mucous membrane and to widespread necrosis of the intestinal wall. The following changes were noted at autopsy: small, punctate, macroscopic hemorrhages; large and small, solitary or multiple ulcers with or without secondary infection; necrosis of the whole intestinal wall; perforation; fistulae; abscesses, and peritonitis. The microscopic changes included capillary dilatation, hemorrhages, stasis, thrombosis, from slight injury to complete necrosis of the vessels, destruction and sloughing of the glandular epithelium, hyalinization of connective tissue and destruction of muscle. Nine cases exhibiting intestinal injury were summarized.

Mathias [8] described a man, 64 years old, having a hypernephroma who was treated with deep x-rays in three series 10 weeks before his death. At autopsy reddening and swelling of the intestinal mucous membrane were found in relation to adhesions to the tumor and the abdominal wall. Microscopically, there were observed sloughed necrotic epithelium and infiltration of the submucosa and muscularis by polymorphonuclear neutrophils.

Fischer [9] added three instances of necrosis of the intestine caused by irradiation. The first case was that of a woman, 53 years old, who had carcinoma of the cervix. Over a period of 49 days she received 1050 X units of x-rays, followed 1 month later by 7288 mg. hours of radium administered over a period of 38 days. Five days after cessation of the radium therapy, the diarrhea, which she had had since the end of the x-ray treatment, stopped and vaginal bleeding commenced. Seventeen days later she died. Autopsy disclosed: ulcerated carcinoma of the portio vaginalis and posterior cervical wall; a rectovaginal fistula; at the left edge of the pelvis beneath an x-ray skin burn, adhesion of a loop of small intestine and friable sigmoid colon; an ulcer in the sigmoid colon, and ulceration of the atrophic and scarred mucosa of the loop of the small intestine.

Fischer's [9] second case was that of a man, 66 years old, who had a large, inoperable tumor of the prostate and was given 1280 X units of x-rays and 1550 mg. hours of radium per rectum, with improvement. Eight and 12 months later, 1820 X and 1280 X units of x-rays, respectively, were given. Sixteen weeks after the last series, marked fever, melena and death with the appearance of urinary sepsis occurred. Autopsy disclosed adenocarcinoma of the prostate, bilateral suppurative nephritis, fibrous peritonitis, a rectal ulcer 6 cm. inside the anus, scarring 3.5 cm. proximal to the ulcer and a mesocolic fistula.

Fischer's [9] third case was that of a woman, 36 years of age, with carcinoma of the cervix who was given an unstated amount of x-ray irradiation over a period of 3 days, with improvement. One month later two more doses of x-rays were given to the parametria. Four months later urinary retention and oliguria were succeeded by severe urinary obstruction and death followed in 2 months. Autopsy disclosed residual carcinoma enveloping the pelvic organs and ureters, acute cystitis, marked hydronephrosis, adhesion of several friable loops of ileum to the abdominal wall, destruction of 40 cm. of the ileal mucous membrane to the ileocecal valve and fibrinous deposit on the under surface of the right lobe of the liver.

of carcinoma of the bladder and six of cervical carcinoma. He concluded that irradiation therapy for malignancy of the pelvic organs may produce marked secondary effects on the rectum.

Experimental Studies

Krause and Ziegler [21] exposed mice to x-rays and found in the animals dying spontaneously marked changes in portions of the intestine, including severe catarrh, epithelial desquamation, abundant bacteria in the lumen and crypts and atrophy of lymphatic tissue.

Regaud, Nogier and Lacassagne [22] directed filtered x-rays to the abdomens of six bitches. Desquamation of the epithelium of the small intestine, atrophy of the stroma of the villi, disappearance of the villi and of the glands of Lieberkühn, atrophy of lymphatic tissue, perforation of the severely affected intestinal loops and greater changes in loops more directly in the focus of rays were observed.

In his work on the effect of roentgen and radium rays in guinea pigs and mice, Fromme [23] demonstrated the most severe alterations in the colon and small intestine.

Denis, Martin and Aldrich [24] irradiated rabbits with massive doses of filtered x-rays. A very severe systemic reaction and death were produced only when some portion of the intestinal tract lay within the area of exposure.

Szegö and Rother [25] demonstrated in dogs that human therapeutic doses did not affect the gastric secretion. Massive doses of x-rays to the stomachs of dogs resulted in progressive cachexia, atrophy of the gastric mucosa and burns of the intestinal mucosa.

In guinea pigs, Keller [26] showed that a smaller amount of x-ray irradiation was required to affect the bone marrow and blood than to injure the intestine.

In a series of papers, Warren and Whipple [27] confirmed and extended the work begun by Hall and Whipple.[28] They demonstrated that a massive dose of x-rays over the thorax (up to 512 ma. min.) produced no evidence of clinical disturbance. This was in contrast to the fatal intoxication due to the injury of the small intestine caused by massive doses (350 ma. min.) of x-rays over the abdomen. During the third 24-hour period after exposure, retching, vomiting, bloody and mucous diarrhea, and tenesmus were observed. The small intestine was red and inflamed. The epithelium had nearly vanished, leaving a collapsed framework of edematous mucosa infiltrated by wandering cells. In the fourth 24-hour period, the peak of intoxication and death preceded by coma took place. Histologically, evidence of mitoses and efforts to repair the intestinal epithelium were seen. They considered the epithelium of the intestine as at least as sensitive to x-rays as the lymphocytes of the same area. They showed also that the normal intestinal epithelium undergoes autolysis much less rapidly than irradiated intestinal epithelium, that no bacterial invasion of the body takes place even after the intestinal epithelium has been destroyed by irradiation, that small, repeated doses of irradiation over an interval of 5 or 6 days give a summation effect, that a cone of x-rays may be used to injure part of the gastro-intestinal tract, and that sensitivity to x-rays varies among animals of different species.

Martin and Rogers [29] exposed isolated loops of small intestine of dogs to 75 ma. min. of x-rays. These animals lived from 3 to 5½ months after operation. Before death they evinced anorexia, weight loss, emaciation and cachexia. The x-rayed loops of intestine showed stenosis, thickening and ulceration.

Ivy, McCarthy and Orndoff [30] exposed dogs with Pavlov pouches to one human erythema dose of x-rays of short wave length. All of them had partial or complete anorexia, hyposecretion of gastric juice and anacidity beginning on the third day and continuing for 2 to 3 days. Recovery or death depended on the vitality of

of 3 feet of jejunum; two partially gangrenous jejunal loops separated by less damaged gut, and hemorrhage into the sacrum. Microscopically the jejunum, ileum and colon showed disintegration and desquamation of the epithelium, hemorrhage into the lumen, atrophy of the lymphatic tissue, fibrosis and an attempt at repair in the completely denuded areas.

Elliott and Jenkinson [15] described a man, 48 years old, who had Hodgkin's disease with general lymphadenopathy, palpable enlargement of the mesenteric lymph nodes, splenomegaly and an epigastric mass. Over a period of 8 months he received 2180 r. in nine series, one to the anterior and one to the posterior abdomen, with 320 r. the maximum dose through any portal. After 2 months, an abdominal mass of the right lower quadrant was discovered. His temperature rose to 104.6° F.; his abdomen became swollen and rigid. A laparotomy showed a fibrinous peritonitis and a swollen appendix which was removed. He died 5 days later. Autopsy revealed the recent laparotomy wound, absence of the appendix, general fibrinous peritonitis and an encapsulated, hemorrhagic perforation on the posterior wall of the stomach. The lymph nodes were enlarged. The stomach contained a dark red blood clot. The posterior surface of the cardia was involved by a large ulcer ringed with satellite ulcers. Two smaller ulcers were on the greater curvature. The ileum showed gray-green ulcerations partially encircling the lumen and distributed from 90 to 200 cm. proximal to the ileocecal valve. The gastric ulcer had a scar and granulation tissue base infiltrated by lymphocytes, monocytes and a few polymorphonuclear neutrophils. The ileal ulcers were edematous and infiltrated by lymphocytes and polymorphonuclear neutrophils. The lymph nodes showed tissue destruction with a slight resemblance to Hodgkin's disease.

Collins and Jones [16] analyzed a series of 422 cases of cervical cancer treated by irradiation therapy. Of these, 6 cases presented the late (4 months to 3 years) complication of benign stricture of the intestine. Five were in the sigmoid colon, 1 in the small intestine. The lesions consisted of an annular fibrous thickening in a localized segment of the intestine associated with varying degrees of constriction of the lumen.

Newell and Crossen [17] listed the complications in the intensive treatment of advanced carcinoma of the cervix by combined roentgen-ray and radium therapy. Five cases of rectal stricture were found.

Todd [18] outlined the case of a woman with cervical carcinoma who received 4000 r. over a period of 4 weeks. During the last week of therapy she developed severe symptoms of enteritis and proctitis and died within 3 weeks. At autopsy patchy ulceration of the small intestine and rectum and histologic necrosis of the mucosa were found. The rectal lesion appeared to be part of a widespread, acute enteritis.

Corscaden, Kasabach and Lenz [19] collected 15 instances of injury to the intestinal mucosa. Among these was an ulcer of the rectosigmoidal colon occurring 2½ months after the end of treatment. At operation the specimen showed necrosis of the entire wall. Another case, 2 months after the end of treatment, disclosed pneumonia, an annular ulcer in the rectosigmoidal colon with microscopic mucosal necrosis, no carcinoma, cloudiness of the submucosal cells and fibrosis of the internal muscle coat with infiltration of plasma cells and polymorphonuclear neutrophils at autopsy. A case of ulceration of the small intestine with onset 2 months after the end of treatment revealed the following at autopsy: fibrinopurulent peritonitis; fibrous adhesions; friable intestinal walls; a thick, hemorrhagic, ileal mucous membrane with microscopic evidence of destruction of all but the crypt epithelium; submucosal edema and inflammation; perimetritis; cervical necrosis, and residual carcinoma.

Ferguson [20] described the lesions in one case of carcinoma of the prostate, one

the physical findings were unchanged. The blood hemoglobin was 11.5 gm. (Newcomer); erythrocytes, 3.97 million per cmm.; total leukocytes, 5350 per cmm.; the differential leukocyte count essentially as before. The stool contained blood (++++).

On August 24th the temperature rose to 101° F., the pulse to 200. Sedation, starch enemas and a high caloric-vitamin diet were employed. On August 27th a transfusion of 500 cc. of citrated blood was given. In spite of these measures, intravenous fluids and a Wangensteen apparatus to relieve distension, the nausea, diarrhea and abdominal pain continued. The temperature ranged between 96° and 102.4° F. and the pulse from 115 to 140. Death occurred on September 3rd at 12:45 a.m., 32 days after admission and 14 days after the last exposure to x-rays.

POSTMORTEM EXAMINATION

Autopsy, performed 8 hours after death, revealed absence of pubic hair, reddening of the labia majora and a sacral decubital ulcer, 8 cm. in diameter.

The body cavities, thymus, thyroid and heart were not grossly remarkable. The intima of the aorta was studded with white and yellow plaques measuring 1 to 5 mm. The posterior portion of the left lung was firm and dark red. The right lung was not unusual. The spleen weighed 90 gm., had a smooth, purple-gray surface and a dark red, firm cut surface with obscure markings.

The esophagus and stomach had intact mucosae and thin walls. They contained a watery, opaque, yellow fluid seen also throughout the gastro-intestinal tract. The coils of small intestine were greatly dilated. The duodenum had a yellow, intact mucosa. The mucosa of the jejunum and of the upper part of the ileum was mottled dark red and yellow. The wall was mottled dark red. The appendix was about 7 cm. long and patent throughout. The thickened sigmoid colon and rectum had yellow mucosae studded with fiery red patches. A 3 mm. polyp projected above the mucosa of the cecum.

The pancreas, adrenals, kidneys and liver were not grossly remarkable. The gallbladder contained two yellow, nodular stones, 1.5 cm. in diameter, had a thin wall, a smooth serosa and a mossy mucosa. The urinary bladder had a slightly reddened mucosa.

The uterus was tethered to the left broad ligament by a firm, nodular band extending to the pelvic wall. The uterus was 9 cm. long. The serosa was smooth. The myometrium, 2 cm. thick, contained a white nodule 1 cm. in diameter in the right cornu. The endometrium was 1 mm. thick, yellow and intact. The cervical canal was stenosed. The external cervical os was enlarged, its surfaces black and its left wall very hard. This induration extended into the left broad ligament. The vagina was slightly wrinkled, gray and mottled with black patches. It contained about 10 cc. of opaque, foul-smelling, brown material. The

the animal at the time of exposure. The pathologic observations bore out the main findings of Warren and Whipple.[27]

Dawson[31] employed 155 to 205 per cent of a dog erythema dose in irradiating Pavlov pouches and found that the order of injury of the cells of the stomach mucosa of the dog is mucous neck, chief and parietal cells. The superficial one-third of the mucosa was disorganized and the regenerated mucosa had about one-half the thickness of normal. The parietal cells may show no histologic evidence of injury, but be unable to produce acid. The Pavlov pouches showed hypochlorhydria followed by achlorhydria.

Podestà[32] irradiated the abdomens of dogs, with heavy filtration. His observations paralleled those of other workers.[27,30]

Wolfer[33] produced chronic ulcers in the stomachs of dogs which were similar, grossly and microscopically, to chronic peptic ulcers in man by exposure of the gastric mucosa to roentgen rays. The ulcers did not cause hypersecretion or hyperacidity and when situated near the pylorus, a definite delay in the emptying time of the stomach was noted.

Tsuzuki[34] administered 100 per cent of a human erythema dose to the backs of rabbits. A severe, general systemic reaction and subsequent death were observed. The gastro-intestinal tract showed hyperemia of the mucous membrane, remnants of destroyed nuclei and degenerated lymphocytes, pronounced degeneration of intestinal epithelium at the bottom of the crypts and destruction of lymphatic tissue with phagocytosis.

Lawrence and Tennant[35] found that the effects of neutrons and x-rays on the whole bodies of mice are very comparable. Animals dying more than 4 days after a lethal dose showed bacterial invasion of the viscera. The intestinal mucosa was ulcerated, acutely inflamed, edematous and gave evidence of regeneration of epithelial cells in the glandular crypts.

Report of Case

A Mexican housewife, 42 years of age, was first seen at Colorado General Hospital on August 2, 1940. She presented the following history: intermittent spotting of blood from the vagina for 7 months; pain in the rectum and bladder aggravated by defecation and micturition for 3 months; low back pain, tenesmus and continuous vaginal bleeding for 2 months. On admission her temperature was 99° F. The other vital signs were normal. The patient was crying out with pain and was critically ill. The significant findings were limited to the pelvis. The cervix was nodular, hard and fixed. The parametrial tissues and the rectovaginal and vesicovaginal septa were extensively indurated. The blood hemoglobin was 12.8 gm. (Newcomer); the erythrocytes, 3.3 million per cmm.; total leukocytes, 8700 per cmm.; polymorphonuclear neutrophils, 74 per cent; lymphocytes, 25 per cent; and eosinophils, 1 per cent. The Wassermann reaction was negative. Biopsy of the cervix (Fig. 1) revealed a squamous-celled carcinoma.

The patient was made fairly comfortable by sedation, analgesia and olive-oil enemas. Between August 7th and August 20th, 4200 r. was given to ten anterior and eleven posterior pelvic fields with a 20 by 20 cm. portal, 215 k.v., 15 ma. Thoraeus filter, 50 cm. skin-target distance, 9 minutes per 200 r., and no more than 200 r. over any portal.

On August 16th, nausea, vomiting and diarrhea began. These symptoms continued (although the rectal pain ceased) until August 21st when they became worse, defecation occurring every 5 minutes through the day. Abdominal pain radiating to the back was experienced. On August 20th the patient had a temperature of 99.6° F., a pulse of 128, respirations of 36 and blood pressure of 142/74. Dehydration, thirst and the general critical condition of the patient were obvious. Otherwise

The bone marrow of the rib had a normal cellularity. A smear showed an almost complete disappearance of segmented, stab and metamyelocyte neutrophil forms with a marked increase in myelocyte and promyelocyte neutrophils and stem cells. Eosinophils and lymphocytes were present in about normal numbers. The erythrocyte line was relatively decreased.

The main anatomical diagnoses were: roentgen-ray intoxication, carcinoma of the cervix (residual, post-irradiation), chronic ulcerative cervicitis with squamous epithelialization, chronic suppurative vaginitis, maturation arrest of neutrophilic granulocytes in the bone marrow.

Comment

The parallel between the clinical symptoms and the pathologic findings in irradiation damage of the gastro-intestinal tract in humans and in animals is readily apparent. The main gap, as in other human diseases, is the difficulty of obtaining tissues, either postmortem or by surgical excision, demonstrating the pathogenesis of the early stages.

Since gastro-intestinal upset commonly follows irradiation of lesions in or adjacent to the abdominal cavity, it is well to remember that an occasional fatality will result, even with doses usually found to be not excessive, such as the 4200 r. employed in my case. The only reported case closely resembling it was that of Todd.[18] This patient also had cervical carcinoma and received 4000 r. Probably more cases of this type than have been recorded have died as a result of irradiation therapy with or without a contribution toward the cause of death by the disease for which the irradiation was used. Probably also the great majority of these cases succumb outside an institution with lessened likelihood of permission for autopsy.

With the use of filtration and several portals of exposure on a deep-seated lesion, skin injury may be relatively slight, but the underlying tissues may be severely irradiated. This is especially disastrous to the intestinal epithelium, which has been considered as sensitive as the lymphocytes in the same area,[27] but less susceptible than the bone marrow and the blood[26] to irradiation. In addition to the immediate destruction of the intestinal epithelium, the more distant effect of irradiation on the blood vessels of the intestine, with thrombosis and infarction of the whole thickness of its wall, must be borne in mind as well as the sequelae of ulceration, perforation, abscess formation, fistulae, peritonitis, fibrosis, stenosis and intestinal obstruction. Similarly, tumor cells may be killed either by their inherent sensitivity to irradiation, or being relatively insensitive, by choking off their blood supply through vessel damage.

In determining the location of x-ray injury to the gastro-intestinal

fallopian tubes and the ovaries were atrophic. The bone marrow was dark red and soft.

The brain weighed 1345 gm., but showed no gross abnormalities.

No evidence of metastasis was demonstrated.

Microscopic Examination

The essential microscopic changes were confined to the gastro-intestinal tract, the genital tract and the bone marrow. The esophagus was well preserved. The stomach showed moderate autolysis of its mucosa. The epithelial covering of the jejunal mucosa was completely absent from the stubby, atrophied villi. The cells of the crypt epithelium were engorged with mucus and showed many mitotic figures (Fig. 3). The mucosal framework was prominent and hyaline streaks coursed through it irregularly. The lymphocyte content of the mucosa was reduced. The lymphatic channels had swollen, hyaline walls. The capillaries were engorged with blood. The colonic mucosa was hyperemic and here, also, the lymphatic channels had swollen, hyaline walls. Small ulcerations (Fig. 4) of the mucosa had necrotic bases infiltrated by a few lymphocytes and macrophages. The floor of the ulcerations was covered with a formless acidophilic material and polymorphonuclear neutrophils. The mucosa of the colon generally was denuded of epithelium and consisted of a thick, mottled, hyaline band infiltrated by small numbers of lymphocytes and macrophages. Occasional giant macrophages were seen. The cells lining the crypts were engorged with mucus and showed many mitotic figures. The submucosa was altered by hyaline deposit and fibrosis. Some of its areas were markedly edematous and were heavily infiltrated by macrophages, some multinucleate. The polyp from the cecum was benign and the overlying mucosa had suffered autolysis.

The hyperemic mucosa of the endocervix was thrown into many papillary excrescences and was heavily infiltrated by chronic inflammatory cells. Dilated, cystic glands were numerous. The mucosa contained blood extravasations penetrated by fibroblasts and areas of fibrosis. Some stretches of the mucosa were covered by a thin, stratified, squamous epithelium. In the wall of the cervix were areas of hyaline necrosis containing bizarre, giant, anaplastic epithelial cells with uneven cytoplasmic borders and huge irregular masses of chromatin indicative of injured nuclei (Fig. 2).

The vaginal epithelium was denuded, laying bare a very vascular granulation tissue crowded with lymphocytes and plasma cells and overlaid by necrotic, hyaline débris and polymorphonuclear neutrophils. In the vaginal wall were large areas of hyaline necrosis including small clumps of tumor-cell ghosts.

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tract, the sites of predilection are those anatomically or pathologically fixed in the path of the x-ray beams. These include loops of small intestine fixed by adhesions, the distal 50 cm. of the ileum, the cecum, the sigmoid colon and the rectum.

Probably the presence or absence of evidences of terminal bacterial invasion of the blood and lymph streams and the viscera is conditioned by both the size of the animal and by the length of time elapsing after death before postmortem examination is performed.

The phenomenon of roentgen-ray cachexia[29] is well explained by the failure of food absorption from the damaged gastro-intestinal tract. Data not included in the record of our patient indicate that she had a ravenous appetite, but was losing weight to an unknown amount (weight at autopsy, 81 pounds) during the course of treatment.

Conclusion

A case of roentgen-ray intoxication is presented, with the typical clinical and pathologic pictures of other reported human cases, particularly those exhibiting symptoms during a first and only course of irradiation directed to or near the abdominal cavity and dying within a few weeks after treatment. The human cases and the experimental studies from the literature dealing with the pathologic anatomy of irradiation damage to the gastro-intestinal tract have been reviewed.

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Irradiation Lesions of Gastro-Intestinal Tract

Mulligan

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DESCRIPTION OF PLATE

PLATE 85

FIG. 1. Squamous-celled carcinoma of cervix, before x-ray treatment. × 130.

FIG. 2. Residual carcinoma of cervix, after x-ray treatment. × 130.

FIG. 3. Detail of jejunal gland with evidence of regeneration of epithelium. × 190.

FIG. 4. Ulceration of colon. × 35.

SYNOVIAL SARCOMESOTHELIOMA (SARCOENDOTHELIOMA) *

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There is a malignant tumor of the synovial membranes which exhibits an apparent pluripotentiality in definitive cellular development, recognized by the presence of both connective tissue and epithelial-like cellular formations. Although Stüer, in 1893, first reported this tumor under the descriptive term of adenosarcoma, it was not until 1910 that Lejars and Rubens-Duval described and interpreted it as a peculiar tumor of the synovial membrane, giving it the name *endothéliome synovial.* The tumor's relatively complex microscopic structure has led a number of subsequent authors to give it various names. Chenot and Tzanck called it an *alveolar carcinoma,* Enderlen a *perithelial sarcoma* or a *sarcocarcinoma.* Smith, after considering the names endothelioma and mesothelioma, chose *synovioma;* Sabrazès and de Grailly (1931) modified it to *synovialoma,* and Wegelin (1928) finally selected the term *synovial sarcoendothelioma.*

REPORTS OF CASES

Case 1

A. B., a female stenotypist, 20 years old, had observed a swelling in the right popliteal space "for some time" (months). Pains, caused by complete extension of the leg and occurring at night, induced her to seek medical attention in the middle of August, 1934. She recalled injuring her right knee by a fall while ice-skating when a child, but this is of doubtful significance. No definite preoperative diagnosis of the tumor was made. X-ray study showed the bone uninvolved. Although attached to the periarticular structures, because of its definite margins the tumor was considered benign and so was locally excised on August 23rd. During the operation the surgeon could find no definite attachment to the knee joint capsule or to the overlying skin and thought that the tumor arose from some deep structure near the popliteal vessels. Healing was prompt.

Pathologic Report on Original Tumor. The tumor, 6 cm. in diameter, had an irregularly nodular surface. Section showed it to be made up of a light gray, granular and fibrous tissue that was firm except for some small areas of softening with hemorrhagic infiltration. A milky juice could be scraped from the cut surface. Histologically the connective tissue stroma was found to be very cellular for the most part. Epithelial-like cells occurred as solid cords, as the lining of large and small spaces and as a regular layer covering connective tissue papillae

* This work was done under the direction of Dr. Hermann Chiari at the University Pathological-Anatomical Institute, Vienna, Austria, while on study leave from the Hahnemann Medical College, Philadelphia, Pa.

Received for publication, July 10, 1941.



Subsequent Clinical Course. Within 3 months there was recurrence on each side of the scar, with limitation of extension of the knee joint. Amputation was advised but the patient declined. At this time there was no loss of weight and no detectable lesions except in the region of the right knee. The tumors grew so rapidly that by January 25, 1936, one nodule was the size of an apple, the other walnut-sized. Both were firm and painless. The overlying skin was livid and perforated by pinhole ulcerations that exuded a bloody fluid. The inguinal lymph nodes were moderately enlarged, firm and painless. X-ray study showed pulmonary metastases and atrophy of the distal end of the femur. Intensive x-ray treatment of both the local growth and the lungs was instituted. The popliteal tumors continued to grow, becoming soft and widely ulcerated over their surfaces. In roentgenograms taken on March 30th the pulmonary metastases were no longer visible. The patient finally consented to radical operation. The right leg was amputated just below mid-thigh on April 8, 1936.

Pathologic Report on the Amputated Right Lower Extremity. Two tumor masses were found in the upper popliteal region, one arising from each side of a widened longitudinal scar. The medial mass measured 13 by 11.5 by 7 cm.; the lateral one, not so large, 8.5 by 6 by 4 cm. Their discolored, ulcerated surfaces were covered with pus; the surrounding skin was thin and adherent to the underlying tumors. A deep extension of the tumor was found between the vascular bundle and the femur, almost to the line of amputation. A median longitudinal section through the femur and tibia showed the combined tumor mass filling the entire popliteal space (Fig. 5), extending to but not involving the femur. The bone was unchanged except for an increased porosity (atrophy). The cut surface of the tumor showed it to be fairly sharply demarcated from the surrounding soft parts. It was formed of a gray-white tissue, for the most part fibrous in texture, divided into large areas by wide connective tissue septa. With the naked eye small cysts could be seen, not exceeding the size of a pepper seed and filled with a colorless fluid, located in the fibrous neoplastic tissue. Superficially there were larger smooth-walled cysts, up to 1 cm. in size, some filled with a brown-red fluid, others by dark red blood clots. Especially in the cranial pole of the tumor and over its ulcerated surface were found irregularly marginated, yellow foci of necrosis and an old hemorrhagic infiltration. Histologically the tumor had the same appearance as that noted in the previous report of June 24, 1935.

Terminal Clinical Course. The amputation stump healed with fair rapidity, and recurrence in the stump could never be determined by clinical examination. Intensive x-ray therapy to the stump and chest was continued at intervals. Large metastases were again seen in the pulmonary roentgenograms on May 19th. Pains in the chest, a continuous irritative cough, dyspnea and finally hemoptysis developed. The patient died on March 16, 1937, after an illness lasting 2 years and 7 months.

Pertinent Postmortem Findings. The skin of the thoracic region was pigmented brown (x-ray therapy). The transverse scar on the distal

(Fig. 1). These cells had a small nucleus, rich in chromatin, and where the cells were arranged in a layer the cytoplasm was small in amount and definitely eosinophilic. With the mucicarmine stain the contents of the cystic spaces stained as true mucus. Sections from the surface showed a distinct connective tissue capsule, in no place broken through by the tumor.

This tumor was originally diagnosed as an adenomatous neoplasm, possibly taking its origin from a dermal appendage. Subsequent discussion between the clinician and the pathologist indicated that this diagnosis was not considered as completely final; it was ventured that it might be a mixed tumor; in any case it was considered benign.

Clinical Course. The patient remained symptomless until the early part of the following year when occasional tearing pains in the leg were noted. By the beginning of May she noticed an enlargement at the site of the previous operation. Clinical examination, on June 17th, showed a tumor 7 by 4 by 3 cm. in size, in the popliteal space, under the upper half of the old scar. The tumor was hard and attached to the deeper structures; the overlying skin was only slightly fixed, but contained numerous, visible blood vessels. Inguinal lymph nodes were not enlarged. This recurrence was removed on June 23rd. As the tumor had to be dissected from the deep vessels the surgeon did not consider it as a radical removal. At operation the appearance of the neoplastic tissue suggested sarcoma. The wound healed directly, allowing complete freedom of motion of the knee joint.

Pathologic Report on First Recurrence. An oval mass of tissue, measuring 6 by 4 by 3 cm., was covered, for the most part, by loose connective tissue and fat (Fig. 2). The cut surface was partly reddish gray, soft and homogeneous; partly that of a finely villous, gray-white tissue that was subdivided into areas of various sizes by delicate connective tissue septa. Here and there the tissue contained small spaces, up to 2 mm. in diameter, filled with a moderately viscid, colorless fluid. The tumor was infiltrated by fresh hemorrhage in numerous places and its central portion was brownish in color. Histologic examination showed that the neoplastic tissue was formed of spindle and oval cells with oval nuclei. Mitoses were frequent. Small blood vessels were numerous. Special staining methods demonstrated a fibrillary interstitial substance between these cells. This tissue was arranged in papillary formations which were covered by epithelial-like cells, which here and there showed a definite cylindrical form (Fig. 3). Reticulin stains showed no interstitial fibers between these epithelial-like cells. Their boundary against the stroma was for the most part sharp; in some places, however, the cells of the stroma appeared to merge with the epithelial-like elements (Fig. 4). Staining with mucicarmine revealed a true mucous substance in the clefts between the papillary structures and in small glandlike spaces lined by the epithelial-like cells. The histologic diagnosis was synovial sarcoendothelioma.

tangentially arranged, but a definite basement membrane was lacking. Moreover, in a few situations the sarcomatous cells merged into the epithelial-like, lining cell-layer with uninterrupted transition. The contents of the glandlike spaces and clefts stained red with mucicarmine. The various nodes had the sharply delimited margin of growth of metastases. Sections from the wall of a large cavity in the left lower lobe showed widespread necrosis of neoplastic and pulmonary tissue with edema and inflammatory infiltration of the marginal tissue of the lung.

The initial impression on cursory examination of these sections was that of adenocarcinomatous metastases. When attention was given to the specialized sarcomatous stroma, the association of it with the epithelial-like structures and the finer cytologic details of both components, the diagnosis of pulmonary metastases of a synovial sarcoendothelioma was readily made, especially in view of the previous findings in the case.

The salient features of this case are the characteristic clinical course of this tumor in the region of the knee joint, with repeated recurrences after operation and ultimate spread to the lungs, and the noteworthy histologic appearance of the pulmonary metastases. The complex structure of synovial sarcoendothelioma found in the original tumor was repeated in both recurrent masses and in the lung deposits.

The rarity of these tumors prompts me to report another case, of which the termination is as yet unknown.

Case 2

(Clinical report through the courtesy of Dr. E. Stumme, Znaim, Austria.) Recent growth in a preëxisting tumor in the popliteal fossa of M. E., a housewife, 56 years old, led to an original diagnosis of malignant degeneration of a "ganglion." At operation on October 22, 1938, the tumor was found to have an ill-defined, infiltrating margin, with attachment to the sheath of the popliteal vessels demanding amputation. After the operation the surgeon's opinion was that he was dealing with a malignant hemangioma.

Pathologic Report. The tumor, isolated from the popliteal fossa of the amputated leg, was irregular in shape, and measured 10.5 by 5.5 by 4 cm. In its interior was found a whitish tissue with areas of hemorrhagic infiltration. The margin was poorly defined, for the tumor invaded the neighboring tissue including the sheath of the popliteal artery. Histologic study of sections from this tumor showed the combination picture characteristic of sarcoendothelioma (Fig. 9). Irregularly shaped clefts and spaces were lined by a tissue that was most epithelial-like. Usually this layer was many cells in thickness; only occasionally was it a unicellular stratum. Mitoses were frequent. These

end of the amputation stump was completely healed. The inguinal lymph nodes were not enlarged. No neoplastic tissue was present in the dissected stump or regional inguinal nodes.

The left lung was partly adherent by fibrous pleural adhesions over the lower lobe and the inferior portions of the upper lobe. In the middle of the left lower lobe there was an oval cavity, 13 by 4 by 4 cm. in size, filled with necrotic neoplastic tissue and ichorous fluid. A similar cavity was located at the base of the left lung on the diaphragmatic surface. Near the apex of the left lower lobe there was a sharply circumscribed nodule, 6.5 by 3.5 by 3 cm. in size, of a gray-white, unusually soft, homogeneous tissue, with areas of necrosis and fresh hemorrhage. Several similar tumor metastases were scattered throughout the remainder of this lung. In the phrenico-costal angle of the right lower lobe there was a partly degenerated tumor metastasis, about 5 cm. in diameter, similar in appearance to that in the left lower lobe. Also throughout the right upper lobes there were a number of smaller tumor nodules. In the middle and upper lobes there were numerous foci of lobular pneumonia. The mediastinal lymph nodes under the bifurcation and along the trachea were succulent and anthracotic, but contained no neoplastic tissue.

The remainder of the postmortem examination, which was complete and included the head, revealed no other tumor deposits. Death was caused by lobular pneumonia complicating the degenerating pulmonary metastases.

Microscopic Examination of the Pulmonary Tumor Deposits. Most of the sections, taken from several nodules, had an adenomatous appearance (Figs. 6 and 7). Round, oval or elongated spaces were lined by an epithelial-like layer of cells. This layer was in some places formed by a single row of cells; in others the lining stratum was multilayered. These epithelial-like cells were columnar or polygonal in form, with uniformly medium-sized, round or oval nuclei that seldom showed mitotic figures or polymorphology and contained only a moderate amount of fine, scattered chromatin. The cytoplasm was clear, eosinophilic and rather sparse. The epithelial-like structures were supported by a sarcomatous tissue that was very variable in amount and irregularly distributed in the various nodules. In some few areas it occurred in large sheets (Fig. 8). It was richly cellular with uniform, rather vesicular, oval nuclei. Few intercellular fibrils were seen in most areas other than the solid sheets, although they could be demonstrated readily by special stains. Mitotic figures were infrequent. The epithelial-like layer was usually definitely demarcated from the subjacent sarcomatous stroma; indeed in some places the supporting cells seemed to be

TABLE I

Reported Cases of Sarcoendothelioma

No.	Author	Year	Age	Sex	Location	Origin		
						Joint	Tendon sheath	Bursa
1	Stüer	1893	21	M	R. elbow	+		
2	Burckhardt	1909	46	M	Knee	+		
3	Lejars and Rubens-Duval	1910	22	M	L. knee	+		
4	Chenot and Tzanck	1912	38	F	L. foot	+		
5	Enderlen	1920	40	F		+		
6	Smith, case no. 1	1927			Thigh		?	?
7	Smith, case no. 2	1927	24	F	Thigh		?	?
8	Smith, case no. 3	1927	35	M	Knee	+		
9	Wegelin	1928	28	M	L. knee	+		
10	Schwamm	1930	13	F	Shoulder			+
11	Tavernier	1930	23	F	Knee	+		
12	Diez	1931	33		R. knee	?	?	
13	Prym	1931	66	F	R. knee	+		
14	Sabrazès, Loubat, de Grailly and Magendie, case no. 1	1932	18	F	L. knee	+		
15	Sabrazès, *et al.* (Baillis and Bonnard), case no. 2	1932	50	M	L. elbow	?	?	
16	Bonne and Collet	1935	25	F	L. knee			+
17	Fievez	1935		F	L. ankle	+		
18	Zwahlen, case no. 1	1935	22	F	L. wrist		+	
19	Zwahlen, case no. 2	1935	16	M	Ankle		?	?
20	Black	1936	36	M	R. hand		?	?
21	Brunner	1936	54	M	R. up. arm			+
22	Knox, case no. 1	1936	22	F	R. elbow	+		
23	Knox, case no. 2	1936	33	M	R. knee	?	?	?
24	Fehr	1937	18	M	R. thumb			+
25	von Verebély	1938	51	M	Knee	+		
26	Jönsson, case no. 1 (#71)	1938	50	F	L. wrist		+	
27	Jönsson, case no. 2 (#72)	1938	20	M	Wrist		+	
28	Jönsson, case no. 3 (#73)	1938	23	F	L. axilla			+
29	Jönsson, case no. 4 (#74)	1938	54	M	L. shoulder			+
30	Jönsson, case no. 5 (#75)	1938	40	M	L. up. arm			+
31	Jönsson, case no. 6 (#76)	1938	53	M	Hand		+	
32	Jönsson, case no. 7 (#77)	1938	70	F	L. thigh			+
33	Jönsson, case no. 8 (#78)	1938	40	F				+
34	Jönsson, case no. 9 (#79)	1938	14	M	L. hand		+	
35	Jönsson, case no. 10 (#80)	1938	29	F	R. 4th toe		+	
36	Jönsson, case no. 11 (#81)	1938	37	F	R. temporal			+
37	Jönsson, case no. 12 (#83)	1938	37	F	R. shoulder			+
38	Jönsson, case no. 13 (#84)	1938	69	F	R. hand		+	
39	Berger, case no. 1	1938	30	M	L. thigh			+
40	Berger, case no. 2	1938	38	M	L. thigh			+
41	Berger, case no. 3	1938	26	M	R. axilla			+
42	Franseen, Simmons and Mallory	1939	71	M	R. knee		?	
43	Albot, Thiebaut, Banzet and Hervy	1939	52	F	R. elbow	+		
44	Fisher, case no. 1	1942	20	F	R. knee		?	?
45	Fisher, case no. 2	1942	56	F	Knee		?	?

formations were in turn embedded in a very cellular sarcomatous stroma in which the fusiform elements had oval nuclei. Intercellular fibers were present but not abundant. Frequently the two varieties of cellular formations merged with one another. In one section the tissue supporting the layer lining the spaces was hyalinized. Many areas were necrotic. With the mucicarmine stain the clefts and glandlike spaces were seen to contain mucus.

Literature

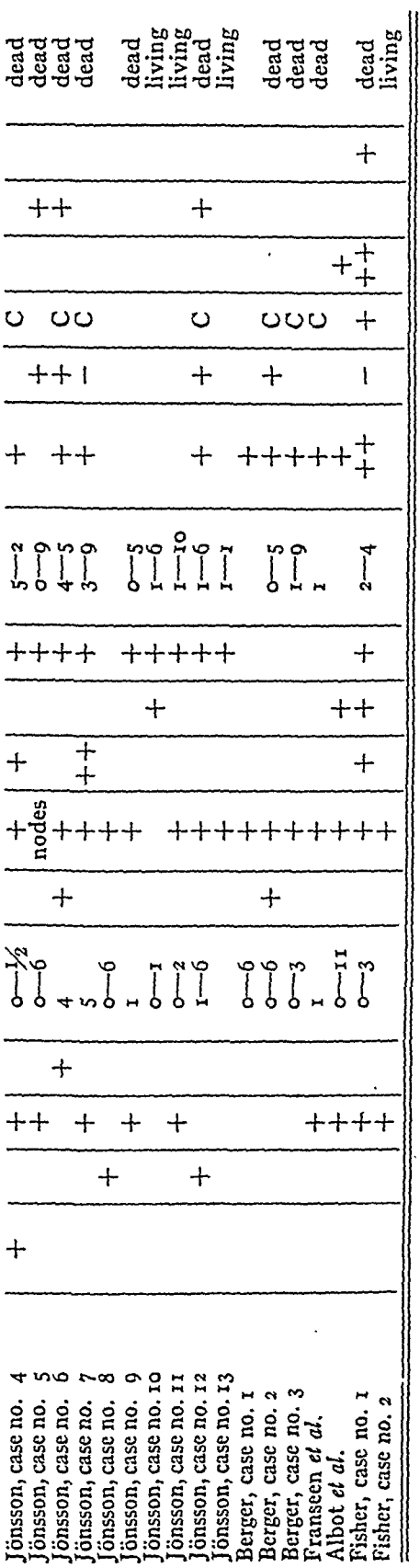
A review of the literature of synovial tumors reveals 43 cases which can be classified as synovial sarcoendothelioma. The criterion for this selection was a bimorphic microscopic appearance, each of these tumors exhibiting both a connective tissue element and a tissue that may be described as epithelial-like, usually found lining small glandlike or cystic spaces or clefts, covering papillary projections or arranged in cords or islands. As a guide in the adjudication of synovial tumors, the descriptions of joint tumors as given by Chiari was followed. The collected cases, with the 2 reported in this paper, are listed in Table I.

In addition to a multiplicity of names, the literature pertaining to sarcoendothelioma is further confused by the previous acceptance of cases which on strict micro-morphologic grounds can hardly be admitted to this group. A number of reviews of this subject have started with a discussion of the older reports of sarcoma of the joints, and continuing into the cases of sarcoendothelioma have drawn no definite line of separation. Frequently the name given a reported tumor is so nonspecific, such as "sarcoma," that the entire case must be reviewed to determine its exact nature.

The connotation of the term sarcoendothelioma has varied with different authors. An illustration to point is the paper by Coley and Pierson in which 15 cases of "synovioma" are reported. According to Smith, who originated the term, synovioma is this peculiar bimorphic tumor with which we are dealing. One might then presume that all of Coley and Pierson's cases were of this variety, but Stewart, who was responsible for at least the majority of the microscopic diagnoses on these tumors, considered the term synovioma to include sarcoendothelioma, giant cell tumor and xanthoma, and sarcoma, when arising from synovial membrane. Giraud, Salmon and Paillas gave synovialoma a similar scope. Geschickter and Lewis classed synovioma with giant cell tumor and xanthoma, and gave it no discussion as an entity.

To save future repetitious bibliographic investigation, the cases not considered as sarcoendothelioma for one of the above reasons will be found in the appended supplementary bibliography.

In 1902, Spencer referred to a sarcoma of the synovial membrane as an endothelioma. During the last decade this name has been applied to a number of cases, and they comprise a group that gives the greatest difficulty in classification. In this category we would place the cases reported by Harbitz, case no. 1, 1927; Wagner, two cases, 1930; Hodgson and Bishop, 1935; von Rosen, 1937 (Jönsson, case no. 82); Coley and Pierson, cases nos. 5 and 10, 1937; possibly that reported by Janik, case no. 5, 1927; and Bogetti's angioendothelioma, 1937. These tumors exhibit elements that have grown as sheets of cells with a fair amount of cytoplasm, little



* C = Clinical evidences only.

intercellular substance and often with an apparently definite cellular orientation. They undoubtedly warrant the name "endothelioma," as indefinitely restricted as this term is occasionally used (Ewing), but it is felt that such a tumor should not be included under the bimorphic synovial sarcoendotheliomas.

A number of cases remain unclassified. The cases of sarcoma reported by Krüger, case no. 2, 1903, and by Julliard and Descoeudres, 1904, might possibly be sarcoendotheliomas; in the latter case, which has been considered a synovioma by some (Jönsson), while the accompanying vague drawing suggests the papillary formation in a sarcoendothelioma, the author stated definitely that the "endothelium" lining the joint and covering the sarcomatous element was unchanged. The case reported by Marottoli (1936) contained one area that suggested a sarcoendothelioma, but the greater part of the tumor had a xanthomatous appearance. In Coley and Pierson's series of 15 cases, which included the 3 cases given by Adair (1935), individual case details were not given. A similar difficulty was met in Jönsson's large series (1938). In his general discussion of synovial tumors, Jönsson described sarcoendothelioma very carefully, but the inclusion of another tumor (case no. 82) in his series of synovialomas cast doubt upon the exact nature of the remainder. Personal communication (1939) definitely established these cases as sarcoendotheliomas. The case reported by Welch, Hampton and Mallory (1938) as synovioma, has Ewing's agreement as to its nature, but Mallory definitely stated that the tumor did not show a mixture of epithelial and spindle-cell elements. Bursell and Gellerstedt (1937) reported a possible sarcoendothelioma which they hesitated to classify definitely, and they pointed out the similarity of these tumors to the chronic hemorrhagic villous arthritis reported by Mandl. Finally, Pack (1939), in the legend to a picture, mentioned a synovioma without giving further details.

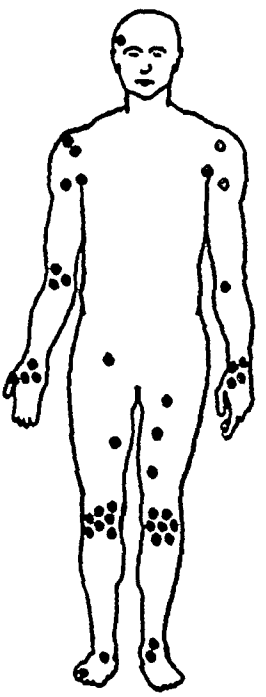
TABLE II

Features of the Reported Cases of Sarcoendothelioma

Case	History of trauma	Initial symptom: Pain	Initial symptom: Swelling	Initial symptom: Limitation of motion	Duration of symptoms before obtaining treatment (yr. mo.)	Treatment: Biopsy	Treatment: Local excision	Treatment: Radical excision	Treatment: Amputation	Treatment: X-ray	Interval from first treatment to termination (yr. mo.)	Local recurrence	Metastases: Lymph node	Metastases: Lung	Bimorphic microscopic structure: Recurrence	Bimorphic microscopic structure: Lymph node	Bimorphic microscopic structure: Lung	Reported termination
Stüer	+	+	++		3		++	+	+			++	+					dead
Burckhardt					6						o—6				+			
Lejars and Rubens-Duval	–	+	+		o—8		+++	+		+	2—5	+++	+		C*	–	–	dead
Chenot and Tzanck									+		2—1				C			dead
Enderlen					o—8		++++			+	4—11	+			C	–	–	dead
Smith, case no. 1	–	+	++		o—5				+			+	+	+	+			dead
Smith, case no. 2					o—3		++++			+		+	+	+	C			dead
Smith, case no. 3		+	+								2—5				–			living
Wegelin				+	5					+	2		–	–	–			dead
Schwamm		+	++		7										C	–		dead
Tavernier					5					+	1—1		+		+			dead
Diez		+	++	+	4										C	–		living
Prym										+	3—6		++		+			dead
Sabrazès *et al.*, case no. 1		+		+	6		+		+		3—5				C	+	+	dead
Sabrazès *et al.*, case no. 2			+	+	1					+			+		C			dead
Bonne and Collet		+		+							3				C	+	+	dead
Fievez					7					+	8—6		+		–			living
Zwahlen, case no. 1		+		+	1—6						1—7				+	+	–	dead
Zwahlen, case no. 2					o—6		++		+	+	1—5		++		–			dead
Black			+	+	1						1—6							living
Brunner					9—3				+	+	4—10		–		C	C	+	dead
Knox, case no. 1		–		+	4						14	+	+					dead
Knox, case no. 2		+							nodes	+	o—4							living
Fehr					o—2				+									dead
von Verebély									+									
Jönsson, case no. 1					+													
Jönsson, case no. 2																		
Jönsson, case no. 3																		

Gross Appearance of the Primary Tumor

The primary tumors varied from the size of a pea (Jönsson) to 20 cm. in diameter (Bonne and Collet). Their consistency was variable; in 16 tumors it was firm, in 11 it was soft, in a few instances the tumor was described as semi-fluctuant or pseudo-fluctuant before it was removed. While occasionally the tumor has been described as an intra-articular polypoid mass (Prym), it is usually a discrete mass located in the soft tissues, and when involving the joint synovia it is



Text-Figure 1. Approximate location of the reported cases of sarcoendothelioma.

customarily placed laterally to the articular surfaces. When examined *in situ* many seem to be well defined tumors, but they often give the impression of being fixed to underlying structures. An apparent capsule has been described in a number of instances. Of the 15 cases in which infiltration was mentioned, 8 had a visibly infiltrating margin. On several occasions the skin overlying the tumor has been ulcerated (Lejars and Rubens-Duval, Smith, case no. 3, Jönsson, case no. 5). The tumors most frequently had a white or gray-white internal color. The microscopic structure of the tumor suggests that small cystic spaces or clefts should often be seen in the cut surface of the tumor, and might be of aid in suspecting the nature of this tumor from the gross examination. Small cysts or clefts were seen by the naked eye in 11 instances: for only 2 was it recorded that cysts were not visible. In 7 of the 11 cases a clear fluid content of the cysts was observed.

I was unable to obtain the original publications of Thomashoff (1896), Vinogradoff (1913), Barbacci (1915), Hohenthal (1934) and Bertini (1936). Bertini considered his tumor to be like that of Wegelin, but the microscopic features were not given in the only review that was obtainable.

Study of the Collected Cases

The features of synovial sarcoendothelioma can be analyzed from this series of 45 collected cases (Tables I and II).

Age and Sex

The age at onset of the first symptom varied from 13 to 70 years, with a median of 35 years. No tumors developed before puberty. From the time of puberty on, the tumor occurs at all ages and with nearly equal frequency (when the age distribution of the normal population is considered). Males and females are affected equally (21 : 22).

Site

The region of the knee joint is by far that most frequently involved, 14 tumors having this location (Text-Fig. 1). The remaining tumors were distributed almost equally in the wrist (3), hand (5), elbow (4), upper arm (2), shoulder (5), thigh (5), ankle and foot (4) and temporal region (1). Fourteen of the tumors arose from a joint capsule, 14 from a bursa, 7 from a tendon sheath; in 7 cases a choice between a tendon sheath or bursa could not be made.

Symptoms

The first symptom is noted in 35 cases. Local swelling was the initial clinical feature in 22 cases; in 11 the condition was heralded by pain. In only 2 cases was limitation of motion of the joint the symptom of onset. Of all the symptoms recorded before the primary operation, pain was noted 21 times, and limitation of motion 9. Seldom was a tumor tender on palpation, as was that of Knox, case no. 2 (1936).

Rate of Growth

Some of the primary tumors had a very slow rate of growth. In 14 instances operation was not performed for 3 or more years; in Fievez's case it was 10 years. Other authors (Fehr, Jönsson) have commented on a primary slowness of growth. This was not the rule in the entire series, for at least 14 were operated upon within the first year. The frequently long duration of symptoms before obtaining medical aid indicates an originally slow growth, and little early disability.

that one is dealing with an epithelial neoplasm until the cellular sarcomatous stroma is recognized. Areas in different tumors have been likened to carcinoma of the breast (Lejars and Rubens-Duval), carcinoma of the intestine and uterus (Chenot and Tzanck), mixed tumor (Schwamm), cylindroma (Smith), hypernephroma (Enderlen) and thyroid tumor metastasis (Wegelin). Brunner's case was first diagnosed an adamantinoma.

Epithelial-like and sarcomatous features are in varying proportions in the same and in different tumors. Since the original observation of Lejars and Rubens-Duval, it has been repeatedly noticed that in some areas one can find the sarcomatous element merging with the epithelial-like formation in such a gradual transition that it is impossible to fix a boundary between the two. These transitions form the strongest morphologic argument for the common ancestry of these dissimilar components.

Recurrence and Metastases

The malignant nature of this tumor is shown by the high rate of recurrence, which was 23 in 30 local excisions that had complete follow-up study. Re-recurrence was noted 4 times. Clinical evidence of spread to the regional lymph nodes was found in 10 instances, 6 of which were confirmed by microscopic examination. Metastasis to the lungs was reported in 22 cases on the basis of clinical study, of which only 3 were confirmed by postmortem examination. Instances of metastasis to the heart and jejunum (Wegelin) and to the spine, clavicle and soft parts (Jönsson) have been recorded.

The recurrent tumors showed the same histologic structure as the original in five cases. In three cases the recurrences were pure sarcoma. The transition of the local recurrences from a sarcoendotheliomatous to a sarcomatous form is nicely shown by von Verebély's case in which the first recurrent tissue exhibited the epithelial-like elements in diminished number; the second showed them completely disappeared, the tumor being purely sarcomatous. In my case no. 1 both recurrences retained the structure of the original tumor. The lymph node metastases in five cases had the bimorphic appearance. In Zwahlen's case no. 2, metastases to the lung were found at autopsy, but no histologic description of them is given. Wegelin expressed the view that secondary growths tend to become more completely sarcomatous, and that the distant metastases contain only sarcomatous elements. On the basis of this single histologically examined case of Wegelin the tendency of pulmonary metastases to assume the sarcomatous form has been accepted by Gruber. My case no. 1 had pulmonary metastases that

Recorded experience indicates that usually the malignant nature of this tumor has not been suspected from naked-eye examination alone. While the gross appearance of sarcoendothelioma cannot be considered as characteristic, this tumor should be suspected when a tumor from an appropriate location has a cut surface that is gray-white and shows very small cysts or clefts that contain a clear, viscid fluid.

Roentgenographic study of the adjacent bony structures has seldom revealed any abnormality, being negative in at least 12 cases. Local atrophy of the nearby bone may occur as a secondary nutritional effect (Fievez, Fisher, case no. 1); direct bony invasion is reported in only 2 instances (Prym, Jönsson, case no 9).

Microscopic Appearance

By original selection each of these tumors had both a sarcomatous element and an epithelial-like tissue, a bilaterality of microscopic appearance that is the essential manifestation of sarcoendothelioma (Lejars and Rubens-Duval). The sarcomatous portion varies greatly, from a rather mature fibrillar tissue to an extremely cellular one in which few fibers are seen between the closely packed oval nuclei. Commonly both extremes are seen in the same tumor. The nuclei are usually only moderately irregular in size and mitotic figures are frequently observed (recorded in at least 18 cases). Occasionally there are areas of large polymorphous cells with large nuclei. There is some tendency to hyalinization (Sabrazès, 1932); and incidental features such as giant cells, xanthomatous cells and doubly-refractile fat droplets (Wegelin, 1928), polymorphonuclear leukocytes (Chenot and Tzanck) and mast cells (Lejars and Rubens-Duval) have been occasionally reported.

The epithelial-like tissue is usually seen lining gland-like spaces, or clefts or small cysts, less frequently as covering papillary projections, or forming solid cords or islands of cells. The cells have a round or moderately oval nucleus in which the chromatin is distributed in fine granules, and nucleoli are not prominent. Mitoses are often seen (noted in at least 11 cases). The cytoplasm is moderate in amount, clear, tends to be eosinophilic and occasionally contains fat droplets and small mucous vacuoles (Wegelin). When lining spaces the cells vary from cylindrical to flattened and are usually in a single cell layer but can be two to three cells thick or have the appearance of a syncytium (Chenot and Tzanck). This lining layer is not always complete. Mucus is always found in the contents of the glandlike tubes and small cysts when the mucicarmine stain is used. In regions where the epithelial-like formations are prominent, the scant connective tissue can appear mature, so that a hasty glance at the section is apt to give the impression

to develop metastases after operation is certainly suggested. This tumor, when operated upon, should be given treatment appropriate for one of very malignant potentialities.

Prognosis

One is struck by the futility of most of the treatment so far recorded. Twenty-seven patients were known to be dead when they were reported; 25 due to the tumor. The other two were Prym's case of "marasmus," Jönsson's case no. 5 of pulmonary tuberculosis. Of the 7 patients known to be living, 6 had a duration of disease under 2½ years. Only Jönsson's patient, case no. 2, living 14 years after treatment, exceeded the customary "5-year cure" limit. It is possible that some benign forms of this condition do occur, as is suggested by Wegelin and Sabrazès. This is maintained by Black. One hesitates to make a dogmatic statement on this small series of cases, for as Jönsson points out this neoplasm is probably more common than the few reported cases would lead one to believe, but with the facts at hand one can give synovial sarcoendothelioma only a most pessimistic prognosis.

The Nature of Sarcoendothelioma

It was originally pointed out by Lejars and Rubens-Duval that the complex microscopic features of sarcoendothelioma could be explained by a consideration of the normal development and histologic structure of synovial membrane. The studies of His, Hagen-Torn, Hammar, Braun, Kroh and Lubosch brought liberation from the old idea of a distinct endothelium lining synovial membranes and developed our present concept of these structures as formed from the development of cavities in the preëxisting mesenchyme, which cavities were then lined by cells derived and only moderately differentiated from the specialized surrounding supportive connective tissue. The lining layer is termed "mesenchymal epithelium" (Jordan; Maximow and Bloom) in deference to its origin. Excellent detailed studies of the minute histologic features of synovial membrane have been given by Key, Sigurdson and Petersen. The differentiated surface cells of synovial membrane do not form a continuous lining layer, and despite their epithelial appearance, evidence of transition from the underlying connective tissue can be found. Cytologic details, like the fine chromatin structures in the nucleus and the relationship of cells to fibrils, indicate their connective tissue source. The growth behavior of the synovial cells is sufficiently individual, however, to lead Vaubel to consider them of definitely specialized morphology, under the name of synovioblasts.

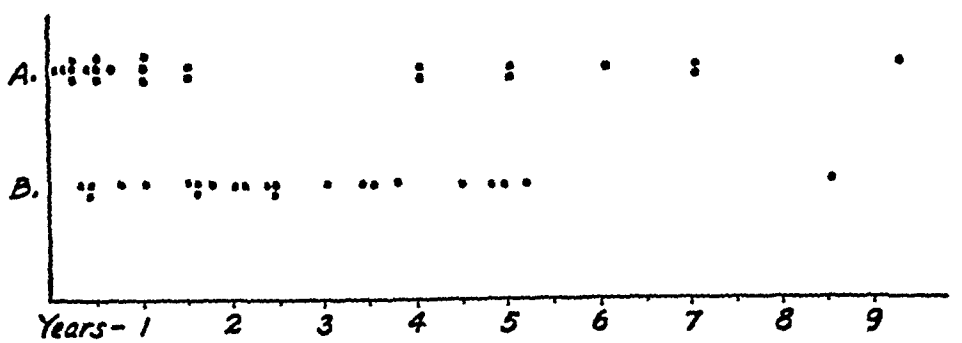
In synovial sarcoendothelioma we have a malignant tumor that in

showed both the epithelial-like and the sarcomatous portions, the former often in preponderance, proving that the pulmonary metastases of synovial sarcoendothelioma need not be exclusively sarcomatous; they can exhibit the same complex structure seen in the primary tumor.

Treatment

Every case was subjected to some type of surgical investigation or treatment. The majority (40) had a primary local excision. Amputation was the first operation in 2 cases. Radical reoperation, amputation, or both, was resorted to because of recurrent growth in 19 cases. Almost all authors commenting on treatment recommended early amputation as preferable (Sabrazès, Zwahlen, Knox).

X-ray therapy was used in 16 cases, usually as an adjuvant measure. Most authors have considered these tumors radioresistant (Fehr, Brunschwig). Jönsson holds the opposite viewpoint, that they are radiosensitive, and his large experience with synovial tumors gives his opinion considerable weight. The phenomenon of the transient disappearance of the shadows of the pulmonary metastases in my case no. 1 following intensive x-ray treatment suggests that these tumors may be temporarily radiosensitive. The term "radiosensitive" is a relative one, however, and no cure through the agency of roentgen therapy has been demonstrated.



Text-Figure 2. Row A gives the intervals of time from onset of first symptom to institution of surgical treatment; row B, intervals from first treatment until death. The mean of the latter intervals for this group of 23 cases is 2.7 years, standard deviation, 1.9.

No untreated patient, with the diagnosis first made by autopsy, has yet been reported, and no reported patient has developed metastases until after operation. These facts raise the question whether the tumor is activated into malignant growth by operative intervention. If this actually happens, there should be an interval of time between operation and death that is relatively constant. This point can be studied in the 23 cases in which sufficient data are given (Text-Fig. 2). The absence of untreated controls prevents any definite conclusion, but a tendency

or synovialoma, but in the present state of our knowledge I feel that this tumor is of such an individually complex structure that it merits its own individual name.

Summary

A bimorphic cellular constituency is considered to be the feature common to a small group of malignant tumors of the synovial membranes. This tumor has been given many names, including synovioma (Smith) and synovial sarcoendothelioma (Wegelin).

Two such tumors are here reported. One of them exhibits recurrent growth and pulmonary metastases which retain the bimorphic microscopic appearance.

From a review of the literature, 43 previously reported cases have been collected; the clinical and pathologic features of this series of tumors are analyzed.

The origin and nature of the tumor are discussed, and, as a compromise between usage and histogenesis, the name *synovial sarcomesothelioma* is proposed.

The salient features of the tumor are: that the tumor occurs with equal frequency at any age after puberty, it is found equally in both sexes and it springs from joint capsule, bursa and tendon sheath locations, most frequently in the region of the knee joint. Its initial clinical feature is usually swelling, but pain is the first symptom in one-third of the cases. The original rate of growth can be very slow. Gross examination of the tumor usually has not suggested its nature. Synovial sarcomesothelioma should be suspected if a gray-white tumor from an appropriate location contains small cysts or clefts. The primary tumor contains both epithelial-like and sarcomatous cellular features; these may be retained in metastases, or metastases may be purely sarcomatous. This tumor is malignant, recurs after local removal and metastases occur particularly in the lungs. There are no reports of such a malignant tumor completing its life history without surgical intervention.

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true neoplastic fashion carries a form of tissue growth to excess. The epithelial-like component can be looked upon as a malignant exaggeration in the differentiation of surface synovial cells. Evidences of its basic nature remain in the coincident sarcomatous proliferation of the supporting connective tissue and in the presence of transitions between these two elements. The occurrence of mucus in the glandlike spaces and cysts of the tumor, demonstrated by the mucicarmine stain, is the neoplastic counterpart of the mucin production studied by Cherry and Ghormley in synovia and by Vaubel in tissue culture.

The Name of the Tumor

Few tumors have been designated by more names than has this one. The original name of *endothéliome synovial* given by Lejars and Rubens-Duval in 1910 was consistent with the general concepts of that time. As related earlier, other appellations were applied to this tumor which were purely descriptive of its complex microscopic picture. The latest, conferred by Albot, Thiebaut, Banzet and Hervy in 1939, was *sarcomes fibroblastiques à type endo-épithélial.* Smith, recognizing the difficulty of using the term "endothelioma" when embryologic and histologic advances had made it inappropriate, and yet wishing to evade the use of any histogenetic name that might be based on knowledge still open to controversy, chose the term *synovioma.* This name was in a large measure satisfactory, as it evaded controversy and yet bespoke an identity. The weakness of such an "organ" name for a tumor is that it can be indiscriminately applied to all tumors from that location, and the 13 years that have passed since the name was first used have seen its exploitation in just this way. Wegelin's name of *sarcoendothelioma* has the advantages that it preserves the strict identity of the tumor and indicates its malignant nature, but the latter half of the name is no longer in accord with the more recent histogenetic concepts. The development of synovial membrane exclusively from mesodermal tissue is a generally accepted fact, consequently, *-mesothelioma* can be substituted for *-endothelioma.* The term, standing alone, would find confusion with the tumors (*e.g.,* of the pleura) that are now called mesothelioma. The prefix *sarco-* has the advantage of definitely indicating a malignant tumor of connective tissue origin. Considering these several features, I would suggest the use of the name *synovial sarcomesothelioma* for the tumor discussed in this paper. This is done with great temerity, for the field is now overburdened with a multiplicity of names. It may be that subsequent investigation will show more features in common between this and other tumors arising from the synovial membranes, and thus increase the justification for the term synovioma,

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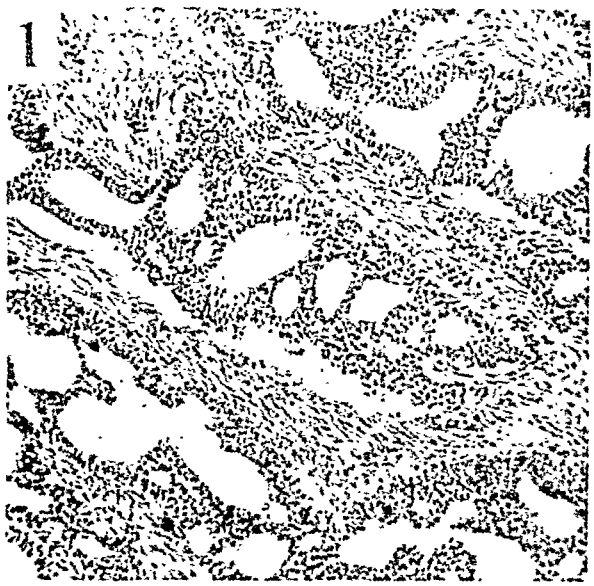
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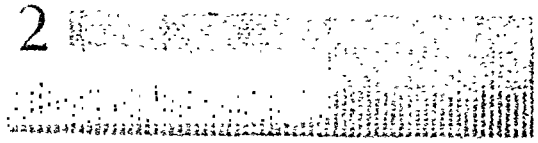
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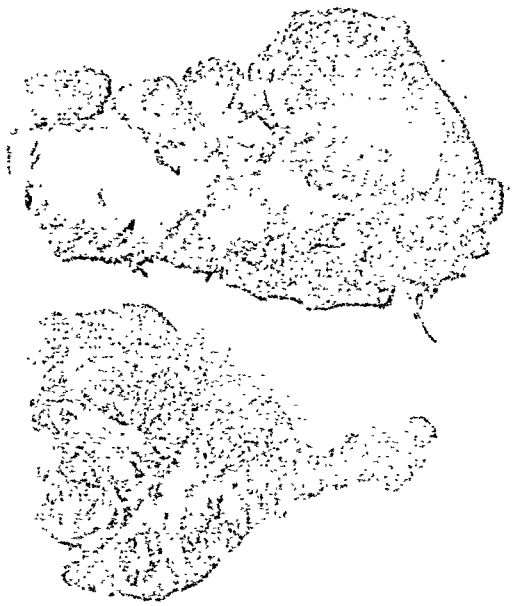
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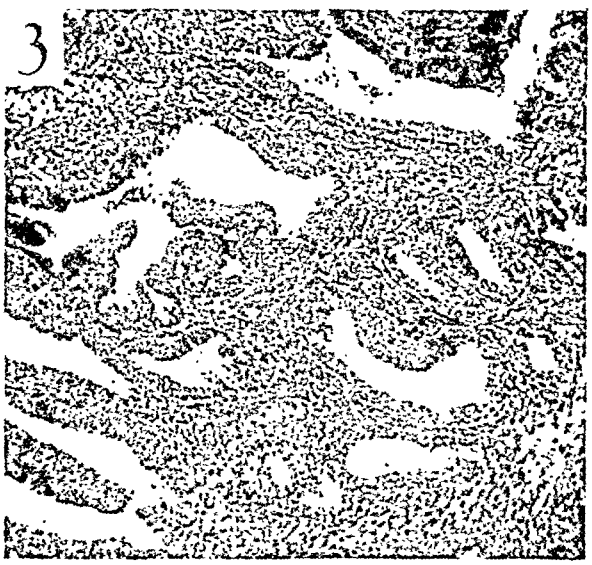
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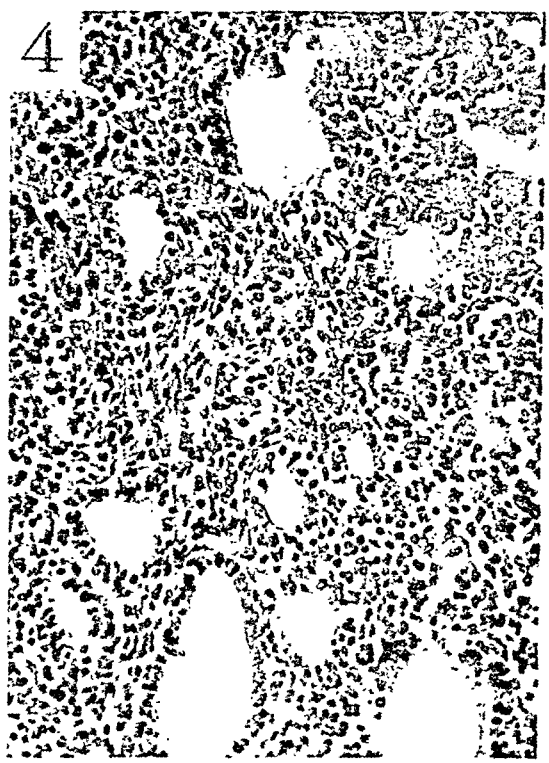
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DESCRIPTION OF PLATES

PLATE 86

FIG. 1. Case no. 1. Epithelial-like formations of original tumor. × 90.

FIG. 2. Case no. 1. First recurrent tumor mass, nearly natural size.

FIG. 3. Case no. 1. Section of first recurrence, showing spaces, papillary formation and lining layer. × 90.

FIG. 4. Case no. 1. The space-lining cell layer occasionally merges with the stroma. × 325.

FIG. 5. Case no. 1. Longitudinal section through the amputated leg showing the second recurrent tumor mass. Greatly reduced.









Fisher

Synovial Sarcomesothelioma

PLATE 87

FIGS. 6 and 7. Case no. 1. Areas of the pulmonary metastases, showing adenomatous forms. × 100.

FIG. 8. Case no. 1. Sarcomatous area in metastasis to lung. × 100.

FIG. 9. Case no. 2. The cells lining the spaces, while differentiated from the stroma, have no definite basement membrane. × 100.

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THE AMERICAN JOURNAL OF PATHOLOGY

VOLUME XVIII JULY, 1942 NUMBER 4

INFECTION OF NEWBORN SYRIAN HAMSTERS WITH THE VIRUS OF MARE ABORTION (DIMOCK AND EDWARDS)*

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As a result of their studies of several outbreaks of abortion in mares in which no cultivable etiological agent could be found, Dimock and Edwards [1-3] demonstrated the infectious nature of the epizootic and in 1932 induced abortion in mares with filtered material from an aborted fetus.

They demonstrated also that a majority of the fetuses revealed a characteristic pathological picture peculiar to epizootic outbreaks of abortion in mares in which no bacterial infection is found. Small, multiple grayish white areas of necrosis were present in the liver which histologically were seen to extend throughout the hepatic substance (Fig. 1) Dimock [3] has described acidophilic intranuclear inclusions in the hepatic cells about the periphery of these foci and in the epithelial cells of bile ducts (Fig. 5). Similar inclusions were found by him in the bronchial epithelium (Fig. 4).

As a result of their studies, Dimock and Edwards concluded that this type of epizootic abortion in mares is due to a filterable virus which induces characteristic lesions in the fetus. Thus far their attempts to induce the disease in animals other than pregnant mares have not been thoroughly successful. The only promising results followed injection of presumably infectious material, filtered and unfiltered, into pregnant guinea pigs. In several instances abortion occurred and all the fetuses were negative on bacteriological examination. No successive transfers were made, and no characteristic pathological changes were described. Because the virus of mare abortion causes a characteristic lesion in aborted foals, the presence of this lesion would serve to establish the diagnosis in the experimentally induced disease. Failure to find the lesion and lack of serial transfer leave the evidence of infection in

* Aided by a grant from the John and Mary R. Markle Foundation.
Received for publication, September 8, 1941.

In our initial attempt to infect hamsters with the virus of mare abortion we tested the possibility of inducing abortion and of transmitting the infection to the embryos *in utero*. A female hamster was inoculated intraperitoneally with 0.5 cc. of a 1:10 suspension of ground liver of an infected foal. Seventy-two hours later six normal young were born, apparently at term. The mother remained well and the young developed normally without any indication of disease. It is likely that 72 hours is too short an interval for infection to become established in the mother and be transmitted to the young, should this be possible in hamsters. Owing to our success in infecting newborn hamsters and the limited number of pregnant animals at our disposal, further attempts to infect *in utero* were not attempted.

Next we attempted to infect newborn hamsters with the virus of mare abortion by inoculating both the mother and the young. A pregnant animal was inoculated intraperitoneally with 0.5 cc. of a 1:10 suspension of ground infected liver from an aborted foal. Four days later four well developed and apparently normal young were born. Hamsters normally deliver 3 to 4 days after pregnancy is detected by inspection. Eight to 14 hours after birth each newborn was given 0.1 cc. of 1:10 suspension of infected liver intraperitoneally. Seventy-two hours later one inoculated animal was missing from the litter. On the ninth day partially eaten bodies of two of them were fixed in Zenker's fluid (5 per cent acetic acid). On the twelfth day the fourth of the litter was missing. Cannibalism of mother hamsters, if their young are diseased or injured, is common. This has presented an annoying problem since the preservation of infected tissue for histological study and for transmission of virus was essential for our purposes.

Microscopical examination of sections of the dead fetuses stained with hematoxylin and eosin showed focal areas of necrosis in the liver and heart (Figs. 2 and 3). Intranuclear inclusions present in parenchymal cells of the liver and in heart-muscle cells (Figs. 6 and 8) were entirely like those found by Dimock in the livers of aborted foals. A similar experiment, without previous injection of the mother, was repeated successfully with two additional litters, the young of which were inoculated intraperitoneally 24 to 48 hours after birth with 0.1 cc. of a 1:10 suspension of ground equine liver. Death usually occurred 3 to 5 days after inoculation and microscopical lesions were found in the liver and heart.

These results indicated the susceptibility of newborn hamsters to the infection and a second serial passage of the virus was established. The liver of a newborn hamster infected with the original horse virus

guinea pigs inconclusive. The same incompleteness of evidence applies to the experimental results of Miessner and Harms [4] and of Hupbauer,[5] although the former investigators stated that they were able to induce abortion in four passages in guinea pigs.

Through the interest of Dimock and Edwards we obtained microscopical sections of tissues and pieces of fresh frozen livers from aborted foals. In the sections we found the characteristic lesions described by Dimock, Edwards and associates, both in the liver and lung.[3] Acidophilic intranuclear inclusions were a prominent feature and gave to the pathological picture a distinctiveness quite convincing of a viral etiology. In addition to the observations of Dimock and Edwards, we have found similar inclusions in nuclei of endothelial cells.

Our first experiments were concerned with attempts to infect chick embryos with bacteriologically sterile, ground liver. The results were entirely negative, as had been previous similar attempts by Dimock and Edwards.[6] In the course of these studies we inoculated with ground liver of aborted foals, pieces of human amnion grafted on the chorioallantois of chick embryos. These inoculations induced lesions in the grafts characterized by the appearance of acidophilic intranuclear inclusions in human amniotic epithelial cells, associated with necrosis, ulceration and inflammation of the epithelial membrane of the grafts. The cellular changes were characteristic and corresponded completely with those found in the hepatic and pulmonary epithelium in the aborted foals. These experiments are described in an accompanying paper.[7] We concluded that human amniotic epithelium, in contrast to the tissues of the chick embryo, is susceptible to infection with the virus of mare abortion, and that the virus is a potential infectious agent for human beings.

Our attempts to infect pregnant and newborn mice and guinea pigs failed. We then made similar trials with newborn Syrian hamsters and these, which proved to be successful, are the subject of the present report. Our evidences of infection in newborn hamsters are entirely histopathological.

Experiments

Dimock and Edwards have sent us, in a frozen state, fresh infected livers of aborted equine fetuses at varying intervals during the past year and a half. These livers have been kept in a carbon dioxide freezing unit at a temperature of approximately $-78°$ C. and have been the sources of virus for our experiments. A fetal liver thus frozen was infective after storage for 7 months. In no instance has bacterial contamination complicated the pathogenic activity of the virus.

one element in the hepatic lesion is especially accentuated, namely, the occurrence of giant cells or syncytia composed of hepatic cells, in which the affected nuclei have undergone multiple division (Fig. 7). The individual nuclei contain inclusions and the mass soon undergoes hyaline necrosis and phagocytosis.

Summary

The chief object of this report is to record our morphological evidence of the experimental transmission of infection by mare-abortion virus through three serial passages in newborn hamsters. Epizootics of economic importance, such as this disease causes, might possibly be controlled by means of vaccination should a suitable method be found. Experimental study in a susceptible laboratory host should facilitate the accomplishment of such an objective.

In the course of our investigations it has been found that the mare-abortion virus is infectious likewise for human amniotic epithelium and in view of this fact one must consider it a potential agent of disease in human beings.

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was removed aseptically. Pieces of tissue were kept for histological section. The remainder of the liver was ground, suspended in saline (1:10) and inoculated intraperitoneally into 11 one-day-old hamsters. Tissues of 6 of these were fixed at intervals between 3 and 5 days. By microscopical examination infection was recognized in each case.

In like manner a third serial passage was established and lesions in the heart and liver were recognized microscopically. In spite of the fact that this virus was lost in the fourth passage, it seems evident to us that successive transmission of infection in newborn hamsters would be uniformly successful if certain precautions were observed. For example, one should determine by microscopical examination that the particular tissues used for the inoculum are well infected in order to insure an infective quantity of virus per unit volume after dilution.

Two nonpregnant adult hamsters inoculated with infected liver have shown no clinical evidence of infection but none has been sacrificed to determine the presence of microscopical foci of infection.

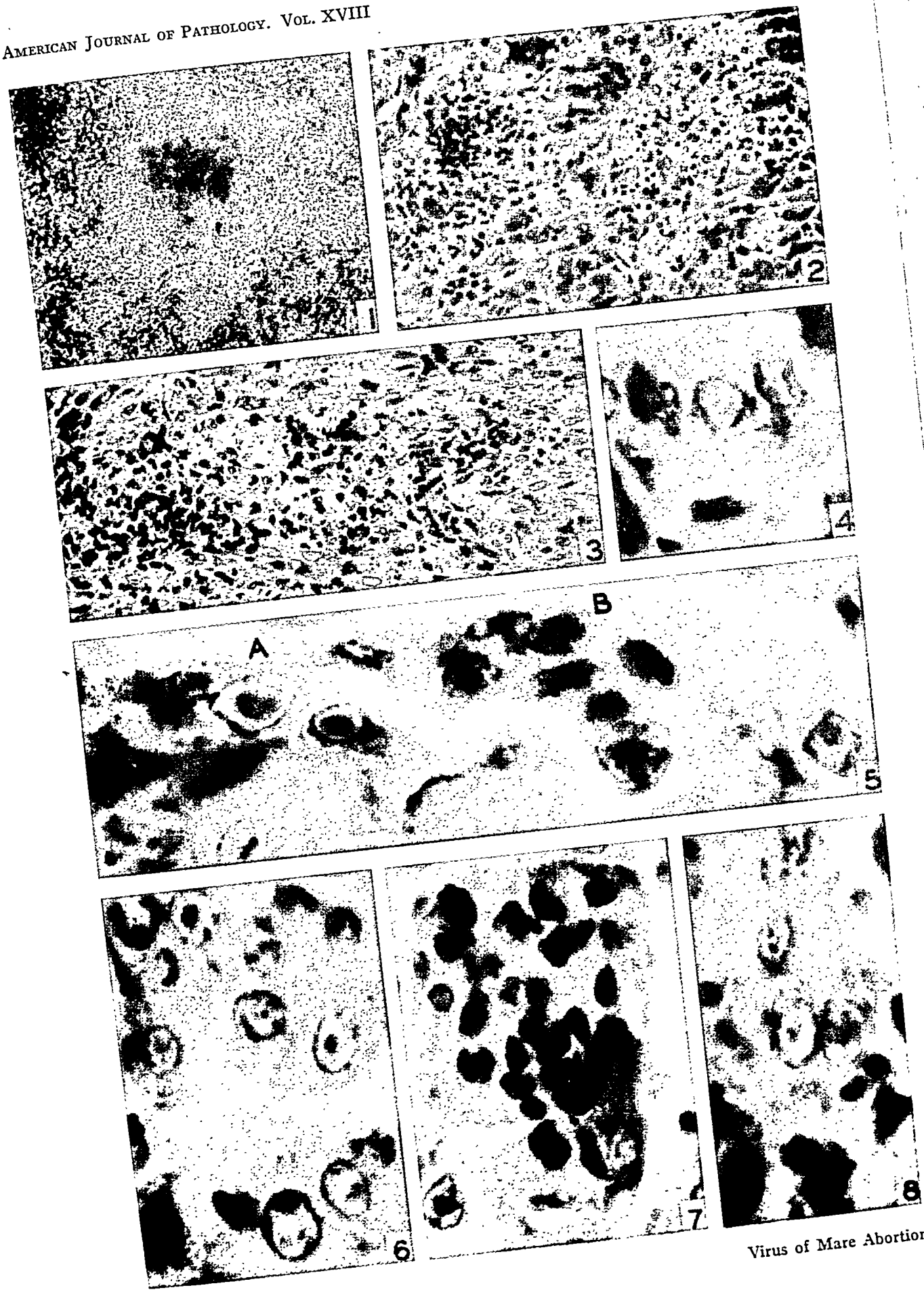
Evidences of Infection in Newborn Hamsters

In equine fetuses dead of infection with the virus of mare abortion, focal areas of necrosis may be found throughout the liver, and petechial hemorrhages in the heart were described by Dimock and Edwards. Histological examination of the hepatic lesions reveals the presence of characteristic acidophilic intranuclear inclusions in parenchymal, biliary and endothelial cells in and about the miliary foci of necrosis. Similar inclusions were described by Dimock [3] as occurring in bronchial epithelium, but no description of cardiac lesions has appeared. The distribution of lesions and the presence of inclusions in endothelial cells indicate a hematogenous spread of the virus in the fetus. No lesion in the placenta of mares has been described although one might suspect its existence as an immediate source of virus extending the infection to the embryo. Such a lesion might well represent a localization of virus from the blood stream of the mare.

Similar foci of necrosis are present in the livers of newborn hamsters dead of infection with the same virus (Fig. 2). Intranuclear inclusions are found quite typically in adjoining hepatic cells (Fig. 6) but we saw none in either biliary epithelium or endothelium. In the hamsters, furthermore, similar areas of necrosis are scattered through the myocardium (Fig. 3), and in and about them nuclei of heart-muscle cells contain the characteristic inclusions (Fig. 8). No lesions and no inclusions have been found in the lung.

The lesions described and accompanying the intranuclear inclusions constitute a characteristic pathological picture. In newborn hamsters

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Virus of Mare Abortion

DESCRIPTION OF PLATE

PLATE 88

FIG. 1. Focal area of necrosis in the liver of an aborted foal infected with the virus of mare abortion. × 46. (Figs. 1, 4 and 5 are from Dimock's sections.)

FIG. 2. Two focal areas of necrosis in the liver of a newborn hamster infected with the virus of mare abortion. A mild inflammatory reaction accompanies the necrosis. × 250.

FIG. 3. Focal area of necrosis accompanied by a mild inflammatory reaction in the myocardium of a hamster infected with the virus of mare abortion. × 250.

FIG. 4. Intranuclear inclusion of the virus of mare abortion in the bronchial epithelium of an aborted foal. × 1200.

FIG. 5. Three parenchymal cells of fetal horse liver (A), showing intranuclear inclusions of mare abortion. Condensed and beaded chromatin is seen at the nuclear walls, and nuclear inclusions surrounded by clear zones. At the lower right corner is another infected parenchymal cell.

Bile duct epithelium (B), containing intranuclear inclusions of mare abortion. × 1200.

FIG. 6. Intranuclear inclusions of mare abortion in parenchymal cells of hamster liver. Three inclusions are well in focus; other cells in the area are infected. × 1100.

FIG. 7. Multinucleated syncytial mass in hamster liver infected with mare-abortion virus. Each nucleus contains an inclusion. There is also an infected parenchymal cell in the lower left corner. × 1100.

FIG. 8. Intranuclear inclusion of mare-abortion virus in heart-muscle cell of hamster. Three other cells in the immediate area contained inclusions not demonstrated at this focus. × 1100.

VIRUS INFECTION OF HUMAN FETAL MEMBRANES GRAFTED ON THE CHORIOALLANTOIS OF CHICK EMBRYOS*

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The possibility of successfully grafting human skin on the chorioallantois of chick embryos was demonstrated by Goodpasture, Douglas and Anderson [1] in 1938. In the experiments reported at that time mention was made of the fact that we had infected such grafts with the viruses of smallpox, vaccinia and herpes simplex. Further experiments were then carried out on the assumption that certain viruses not hitherto proven to be infectious for non-human hosts might infect a graft of human skin surviving upon chick embryonic tissue. Grafts of human skin, both from adults and, in a few instances, from children (removed for plastic operations), were inoculated with material from vesicles of the eruptions of varicella and herpes zoster, and from nodules of molluscum contagiosum. In no instance was there evidence of infection. As a result the questions naturally arose as to whether these particular skin grafts were from persons possessed of acquired immunity to the viruses employed or, if originally susceptible, whether they had become resistant because of an influence from the non-susceptible chick embryo. It was possible to submit both of these questions to experimental test and to obtain at least a partial answer, as will be described.

Our lack of success in obtaining adequate evidence of infection of human skin with vesicular material from lesions of varicella and herpes zoster led to the use of human embryonic tissue, namely, amnion and chorion, for similar experiments. Human placentas were received directly from the delivery room; the fetal membranes from an area near the placental attachment, where they were thin and semitranslucent, were stripped apart and each was spread out with the epithelial surface upward. Grafts about 1 cm. square were placed upon the chorioallantois with the epithelial surface exposed. These grafts became attached readily. The amniotic grafts were successfully inoculated with the viruses of herpes simplex, vaccinia and smallpox but in no instance did evidence of infection result from the use as inocula of vesicular contents and ground vesicular epithelium from the lesions of varicella and herpes zoster.

At the time we were also attempting to find a susceptible host for

* Aided by a grant from the John and Mary R. Markle Foundation.
Received for publication, September 8, 1941.

is no susceptibility acquired from grafting upon a susceptible host. This is illustrated by our failure to infect human amniotic epithelium grafted upon the highly susceptible chorioallantois with the virus of fowlpox although the infected chick chorionic epithelium impinges upon the human amniotic cells (Fig. 4). There is reason to suppose, however, that human skin from a nonimmune individual and amniotic epithelium from human fetuses would be susceptible to the viruses of the human diseases, varicella and herpes zoster; and at present we are at a loss to explain our negative results.

An interesting fact, which concerns the relative susceptibility of amniotic as compared with chorionic epithelium to infection with the viruses of herpes simplex, vaccinia, variola and mare abortion, has appeared in our experiments with grafts from human fetal membranes, in that the amniotic epithelium is readily infectible while that of the chorion has remained, except the grafts from one placenta out of six, uninfected.

Little is known about these tissues in respect to infection and immunity, and the question of a difference in susceptibility to viruses will be the subject of further investigation. It is possible, of course, that the chorion, being immediately in contact with maternal blood or tissues, possesses a high degree of passive immunity conferred by the mother. Presumptively, however, this would hardly account for a difference in susceptibility to the virus of mare abortion; but as yet we know nothing about the nature and distribution of this virus except as an agent of equine infection.

Because the technic which we have used in making, inoculating and studying grafts of the human fetal membranes is of interest, it will be described in some detail.

Technic

Placentas are received directly from the obstetrical operating room in a sterile pan covered with a sterile towel. With sterile instruments an area of thin membrane is located in the neighborhood of the placenta. A sheet from 5 to 8 cm. square is cut out with scissors and spread upon a previously prepared sterile block of cork. Slabs of cork about 10 by 15 by 3 cm. are wrapped in gauze and paper and sterilized. Just before using the wrapping is removed and the gauze moistened with sterile saline solution. The gauze is then folded back, exposing the cork surface, and upon this the sheet of membranes is spread with the chorionic surface upward, if that membrane is to be used for grafting. Upon the exposed surface of the chorion lies a layer of decidua which is first stripped off with forceps. There remains a thin sheet of chorion and underlying amnion. These can be easily separated with the aid of forceps. The sheet of chorion is folded upon itself with the epithelial surface enveloped until used.

The chorionic membrane is then spread out on the cork with the epithelial surface uppermost and with a sharp scalpel it is cut into 1 cm. squares, as many as needed.

the virus of mare abortion which results in considerable loss to stock breeders.[2] Inoculation of chick embryos and their membranes had not been successful and it appeared that the tissues of the developing egg were naturally insusceptible to this virus. We proceeded to inoculate grafts of human fetal membranes with the virus of mare abortion. The result was successful infection of human amniotic epithelium but failure to infect human chorion. This successful experiment demonstrated in the first place the susceptibility of human tissue to infection by the abortion virus, being the first demonstration of infection by it in unnatural host tissues;[3] and, in the second place, that, although the chick chorioallantois is itself not susceptible, that fact did not render the alien graft refractory.

It appeared that our failure to infect human fetal membranes with material from varicella and herpes zoster was not due to a deleterious influence exerted upon the grafts by the insusceptible chick embryo. The possibility remained that the human tissues were perhaps derived from persons who possessed an acquired immunity to these infections and factors responsible for such induced resistance had been carried over with the graft in sufficient amount to prevent infection. All of our experimental experience, however, has been opposed to such an inference. For example, we [4] have recently reported experiments concerned with grafting upon the chorioallantois skin from chickens immune to fowlpox. While a part of the immune fowl, this skin completely resists infection with fowlpox virus by direct inoculation, yet when it is removed and grafted upon the chorioallantois it becomes susceptible again and remains so when regrafted upon muscle of a normal chicken; but it rapidly regains a refractory state when regrafted upon the muscle of the immune chicken from which it was removed. This indicates that acquired resistance of epithelium is soon lost when it is removed from the immune animal and grafted on the chorioallantois. This conclusion is further substantiated by a few experiments with human skin grafts from persons immune to vaccinia. The epithelium from persons with an immunity acquired through vaccination can become susceptible to infection with vaccinia virus when grafted upon the chorioallantois of chick embryos.

We can conclude at the present time only that the human skin and fetal membranes which we have used possessed a degree of natural immunity that prevented infection of their epithelia with the viruses of varicella and herpes zoster, or that other limiting or inhibiting factors, concerning which we are at present ignorant, intervened.

Our experience thus far has shown that at least in the case of apparently complete refractoriness of epithelium to a particular virus there

tulation and ulceration ensue. The inflammatory reaction of the embryonic tissue is quite like that of the natural host.

Infection of amniotic grafts can be induced by direct application of fragments, or of a paste, of infected tissue to the exposed epithelial surface. Intranuclear inclusions occur in abundance; there is some hyperplasia; and infected multinucleated giant cells are at times conspicuous. With ensuing necrosis cellular reaction of inflammation and ulceration takes place (Figs. 7 and 8).

Although infection of amniotic grafts has followed inoculation with herpes virus quite consistently, this has not been the case following inoculation of chorionic grafts, either after direct application of virus, or after snipping the chorion. In fact, in only one series among numerous attempts with grafts from placentas has infection been demonstrated. In this case care was taken to strip off as much of the outer layer of decidua as possible before the grafts were cut (Fig. 9). Great care in this respect was taken with these grafts and because they were the last of the series, we cannot say with what frequency one might expect successful infection if this precaution is always observed. However, we believe that it is much more difficult to infect the chorion with the several viruses which we have used, than it is to infect the amnion.

In the successful series mentioned we also grafted thin sheets of stripped decidua. Sections of these showed much necrosis but no evidence of herpetic infection. The grafts did not survive well.

Infection of Grafts of Human Tissue with Vaccinia Virus

Inoculation of grafts of human skin with vaccinia virus following snipping gives rise to epithelial hyperplasia, Guarnieri bodies, pustule formation and ulceration. Infection of adult-skin grafts occurs quite regularly, even when skin from a person immunized by previous vaccination and retested for immunity is used. In these instances the grafts were allowed to remain on the chorioallantois at least 2 days before inoculation, and it is assumed that within this time any effective immune bodies were diluted by the plasmatic circulation so as to render them ineffective. Similar experiments have already been reported by us [4] in the case of skin grafts from chickens immune to fowlpox. The grafts became susceptible to fowlpox infection under these circumstances.

We have succeeded likewise in infecting human amniotic grafts, but the amniotic epithelium seems to be somewhat more resistant than cutaneous epithelium. Discrete, compact, basophilic, often multiple Guarnieri bodies are formed in the infected cells, and necrosis proceeds rather slowly (Fig. 10).

Eggs, incubated 9 to 11 days, should be prepared beforehand for opening by cutting a square window through the eggshell and coating the surface with melted paraffin.[5] They are then placed in an incubator with window up until ready for use.

The window is opened by cutting the shell membrane on three sides with a half spear-point needle and tearing off the dislodged shell from the fourth side. A puncture through the shell overlying the air-sac facilitates the dropping of the exposed membrane. A square of human membrane to be grafted is adjusted to the flat end of a small, sterile, searing iron of polished steel, with mesodermal surface exposed. The graft is then placed on the chorioallantois, dislodged from the searing iron and smoothed into place (Fig. 1). The window is closed in the usual way with a vaseline-paraffin ring upon which a coverglass is laid. The grafts are fixed at desired intervals by cutting out the chorioallantois to which they are attached, placing it on moist, absorbent paper and fixing in Zenker's fluid.

The procedure is the same when amniotic grafts are desired. The membranes are placed on the cork always with the surface that it is desired to graft upward. The two membranes are then separated, but that which is downward with the epithelium in contact with the cork is always discarded.

Bacterial contamination of grafts from placental membranes prepared in this way has been observed only very rarely.

Description of Grafts

The human membranal grafts readily become attached to the chick chorioallantois and survive throughout the period of incubation, under favorable circumstances. Both chorionic and amniotic membranes are very thin and avascular, and nourishment takes place apparently by plasmatic circulation, for there is no vascularization. Amniotic grafts appear in microscopical sections as a single layer of cuboidal or low columnar epithelial cells resting upon a hyaline basement membrane (Fig. 2). Beneath the basement membrane is a delicate layer of collagen containing rare nucleated cells. The chorionic layer is thicker. Its epithelial surface is composed of several thicknesses of its polygonal cells that rest upon a hyaline membrane, beneath which is avascular collagenous tissue containing few cells but relatively more than the amnion (Fig. 3).

Inoculation is usually performed about 24 to 48 hours after grafting. A paste of virus-containing tissue is applied directly to the presenting surface of the graft without scarification. The technic for making skin grafts has already been described.[1]

Results of Inoculations

Infection of Grafts of Human Tissue with Herpes Simplex Virus

In order to inoculate grafted human skin successfully it was necessary to snip the graft at several places with the points of sharp iris scissors and then to rub into these a fragment of infected tissue. In this way uniform infection of human skin grafts, inoculated 2 to 4 days after grafting, can be induced. Typical intranuclear inclusions occur in the infected epithelial cells, and sometimes vesiculation, pus-

the susceptibility of human fetal tissue to this virus, but also that a susceptible tissue grafted upon the non-susceptible chorioallantois of the chick embryo does not thereby lose its susceptibility. Likewise an insusceptible tissue when grafted upon the chorioallantois does not become susceptible to a virus that readily infects the latter. This was shown by grafting human amnion onto the chorioallantois and inoculating it with fowlpox. Although the hyperplastic, inclusion-containing epithelium of the chick membrane impinged directly upon that of the amnion, no evidence of infection of the latter was discernible (Fig. 4).

Discussion

These experiments have demonstrated the possibility of studying infection of the component elements, amnion and chorion, of human fetal membranes by means of grafting them upon the chorioallantois of the developing chick embryo. Similar experiments have been successfully performed with grafts of human skin.

Little or nothing is known otherwise about the susceptibility and relative susceptibility of fetal membranes from the human embryo, although clinical observation has discovered intra-uterine infection of the human fetus by a number of viruses, notably those of variola, chickenpox and measles. The mechanism or pathogenesis of transmission of a virus from the mother to the fetus through the placental barrier is unknown; and when occasion arises this should be the subject of special study. A beginning in this direction is possible through the indirect method of grafting.

Although our experiments are not sufficient to warrant a definite conclusion, they at least suggest that chorionic epithelium is less susceptible to the viruses used than is that of the amnion.

Because it is well known that in the case, at least, of mothers immune to measles the placenta contains much antibody,[8] it is probable that more antibody is contained in the chorion and adnexed decidua than in the amnion; and the presence of antibody might to some extent increase the resistance of grafts from these tissues. An analysis of antibody content of the three tissues, amnion, chorion and decidua, so far as we know, has not been made.

If the amount of antibody is small, it will probably soon lose its effectiveness in grafts of immune tissue (skin) as has been demonstrated by Goodpasture and Anderson [4] for fowlpox, and as we have shown to be the case with skin from persons immune to vaccinia.

Our experiments with the virus of mare abortion have demonstrated the susceptibility of human amniotic epithelium to this virus; it is therefore a potential agent of human disease. We have likewise shown

In only one instance, and that to a minimal extent, have we succeeded in infecting chorionic epithelium with vaccinia virus. This occurred in the same series of grafts in which herpetic infection was established, and in that series especial care was taken to strip off all of the outer coat of decidua.

Infection of Grafts of Human Tissue with Variola Virus

A strain of variola virus was isolated by Buddingh,[6] in 1938, from a mild outbreak of smallpox by inoculating the chorioallantois with pus from the cutaneous eruption. This strain has since been maintained continuously by membranal passage.

With material from one of the early passages, human-skin grafts were inoculated after snipping. Extensive infection resulted, with the formation of pustules. Cutaneous epithelium in the neighboring tissue contained an abundance of typical Guarnieri bodies. No characteristic nuclear changes or intranuclear inclusions were observed either in the human tissue or in chick embryonic cells (Figs. 5 and 6).

After maintenance in the embryonic membranes for more than 2 years, this strain of variola was used to infect human amniotic grafts. Typical Guarnieri bodies were formed, followed by necrosis, inflammation and ulceration. No typical nuclear changes were seen (Fig. 11).

In a single attempt with several grafts of chorion from one placenta no infection was induced. Here again the resistance of human chorionic epithelium seemed to be greater than that of the amnion.

Infection of Grafts of Human Amnion with the Virus of Mare Abortion

A certain type of abortion of mares was demonstrated by Dimock and Edwards [2] to be caused by a filterable virus. Abortion could be induced in mares by the injection of a filtrate of infected tissue from an aborted fetus. In cells of aborted fetal liver and lungs acidophilic nuclear inclusions were abundant and characteristic.[7]

With emulsified infected liver from aborted foals supplied by Dr. Dimock we have been able to infect epithelial cells of human amniotic grafts from four different placentas. Not all of a dozen grafts from a single placenta showed infection, but some of them from each case presented epithelial cells containing typical nuclear inclusions, associated with necrosis of others and ulceration with inflammatory exudation (Fig. 12). In no instance in grafts of chorion from the same human membranes was infection of chorionic cells observed.

Because embryonic cells of the developing egg are not demonstrably susceptible of infection with the virus of mare abortion, successful infection of human amniotic grafts has served to demonstrate not only

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that in respect to this virus, at least, the grafting of a susceptible tissue upon a non-susceptible chorioallantois does not render the graft insusceptible. Nor did an insusceptible tissue (human amnion) become susceptible to a virus (fowlpox) when grafted upon a susceptible chorioallantois.

Notwithstanding the fact that we have met with no success in our attempts to infect grafts of human skin, amnion and chorion with material from lesions of varicella and herpes zoster, we believe that further attempts should be made not only with material from these infections, but from other virus diseases.

A few preliminary experiments concerned with the application of theelin to human amniotic grafts gave some indication that the epithelium was better preserved and more active than otherwise. Further investigation of the use of hormones would be necessary, however, in order to estimate their effects upon prolonging the life and activity of grafted tissues and upon the susceptibility of their cells to viruses.

Summary

1. Human amnion and chorion have been successfully grafted upon the chorioallantois of the developing chick embryo.

2. Grafts of human skin, amnion and chorion were successfully infected with the viruses of herpes simplex, variola and vaccinia. The lesions induced were typical.

3. Human amniotic epithelium of grafts on the chorioallantois were infected with the virus of mare abortion.

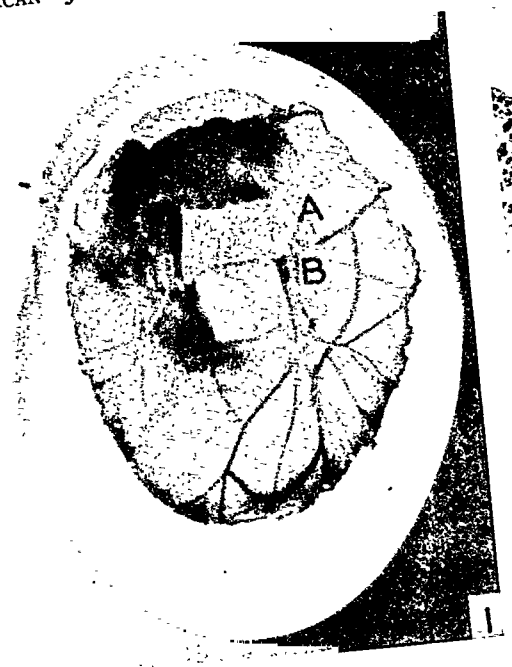
4. It is concluded that a susceptible tissue grafted upon an insusceptible chorioallantois does not thereby lose its susceptibility; and an insusceptible tissue grafted upon a chorioallantois that is susceptible to a certain virus does not thereby become susceptible.

5. By means of grafts of fetal membranes a beginning may be made in a needed study of the pathogenesis of intra-uterine virus infections.

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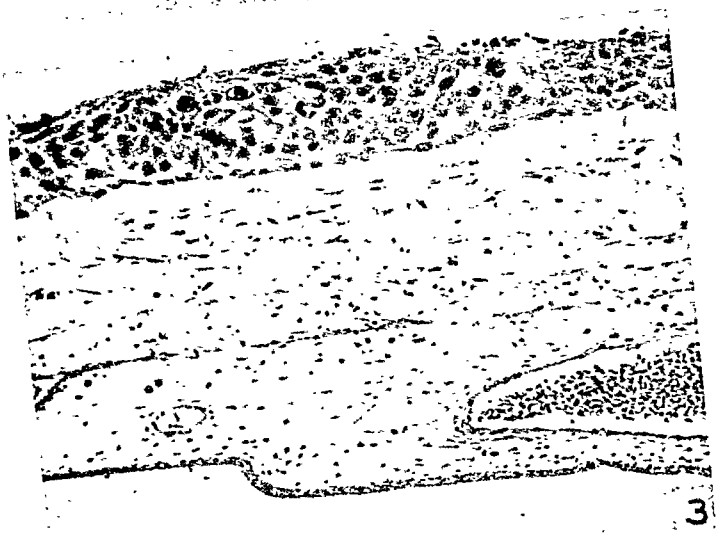
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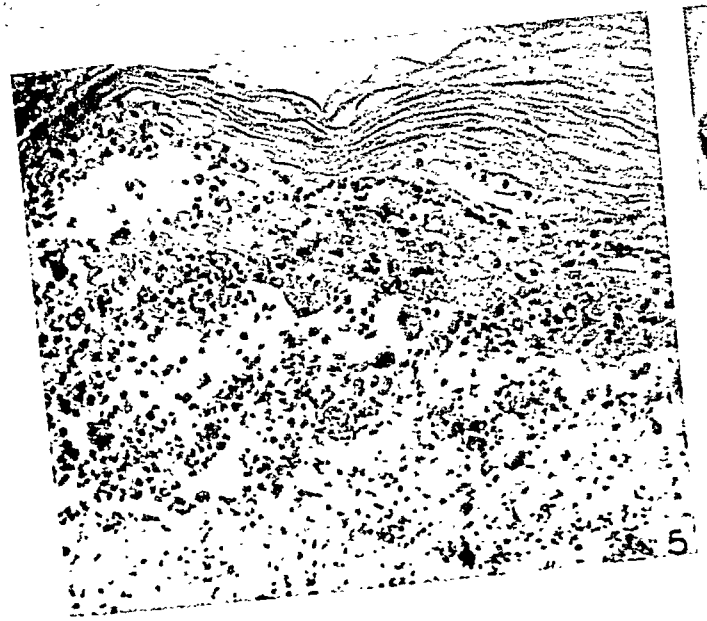
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Virus Infection of Human Fetal Membranes

DESCRIPTION OF PLATES

PLATE 89

FIG. 1. Human amnion (A) and chorion (B) grafted on the chorioallantois.

FIG. 2. Microscopical section of human amniotic graft (7 days). × 120.

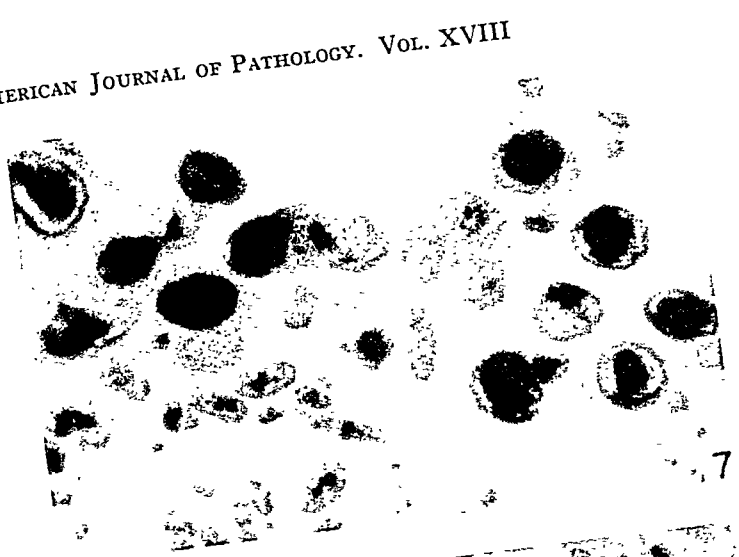
FIG. 3. Microscopical section of human chorionic graft (8 days). × 120.

FIG. 4. Human amniotic graft (left) with its epithelium overlying chick chorionic epithelium infected with fowlpox. The amniotic epithelium is naturally resistant. × 325.

FIG. 5. Pustule in graft of human skin infected with the virus of smallpox. × 225.

FIG. 6. Margin of Figure 5 showing Guarnieri bodies in cutaneous epithelial cells. × 1400.

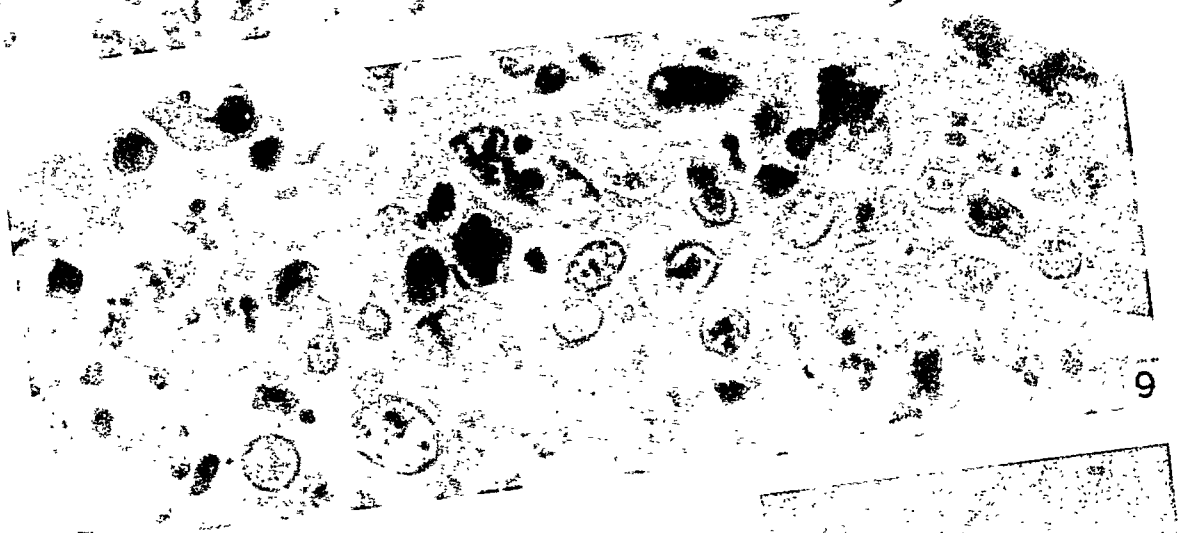
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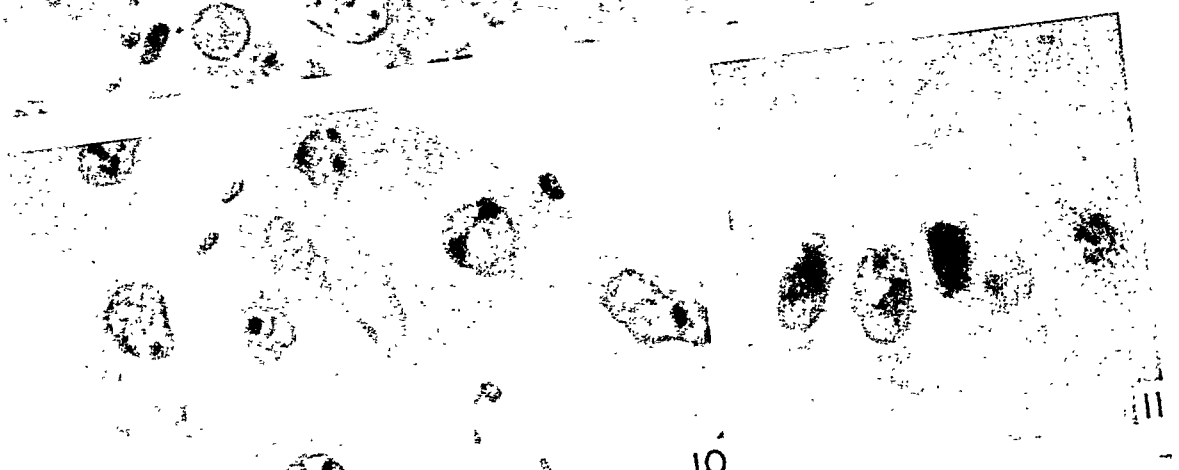
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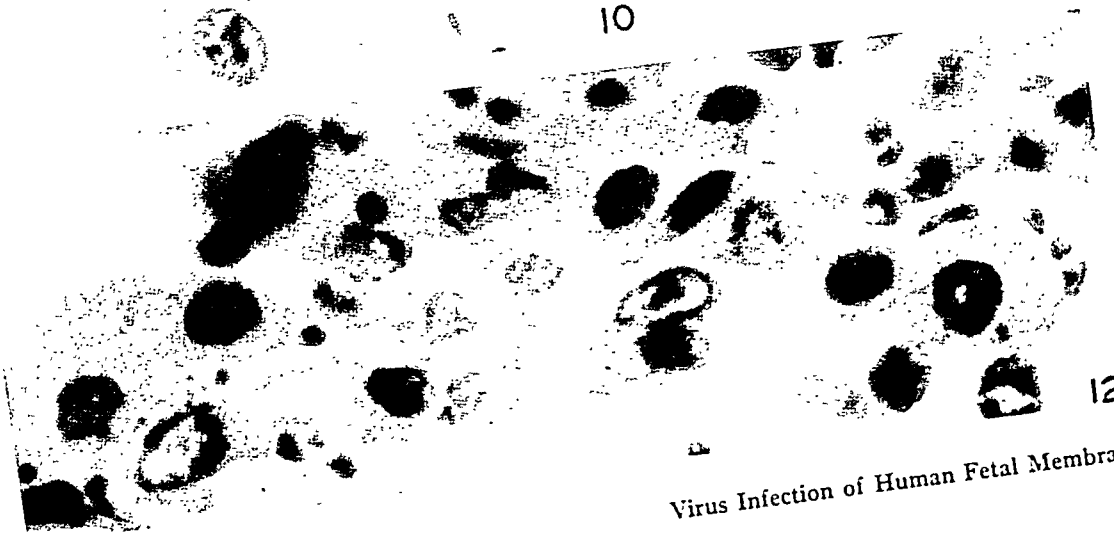


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PLATE 90

FIG. 7. Human amniotic epithelium of graft infected with the virus of herpes simplex. Intranuclear inclusions may be seen. × 1100.

FIG. 8. Same as Figure 7, to show multinucleated giant cell of amniotic epithelium infected with the virus of herpes simplex. Intranuclear inclusions may be seen. × 1400.

FIG. 9. Human chorionic grafts infected with the virus of herpes simplex. Intranuclear inclusions are present. × 600.

FIG. 10. Human amniotic graft infected with vaccine virus, showing Guarnieri bodies in epithelial cells. × 1100.

FIG. 11. Human amniotic grafts infected with the virus of smallpox. Guarnieri bodies are present in epithelial cells. × 1600.

FIG. 12. Human amniotic graft infected with the virus of mare abortion. Intranuclear inclusions may be seen, some of which fill the nucleus. × 1200.

DUAL VIRUS INFECTION OF SINGLE CELLS *

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The hypothesis that activity of one infectious agent within a host may modify the course of disease instituted by another agent in the same host has long been proffered. Such an idea was hypothecated by Edward Jenner [1] after he had observed that the clinical course of vaccinal infection may vary with concurrent herpes simplex. The obligately intracellular parasitic nature of viruses makes the demonstration of multiple viral infections in single cells of some theoretical interest.

The following experiments establish the possibility of dual infection of single cells with different combinations of several viruses. Infections with the viruses of fowlpox, laryngotracheitis of fowls, vaccinia, herpes simplex and rabies are associated with the presence of specific intracellular inclusion-bodies.[2,3] Dual infection of single cells with certain combinations of these viruses have been determined by microscopical recognition of such specific inclusions. This concrete evidence of the activity of two viruses within one cell as presented by the occurrence of two different and characteristic kinds of inclusions in that cell establishes cytological evidence that single cells can become infected with more than one virus. These observations concur with those of Syverton and Berry [4,5] in which they describe coexistent infections of individual cells. These investigators induced specific inclusions of two viruses (herpes simplex and vaccinia) in single cells of rabbit's cornea.[6,7]

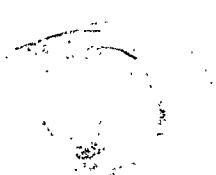
EXPERIMENTAL PROCEDURES

Technic

Chick embryos, 11 and 12 days old, served as experimental hosts. With the exception of one experiment in which nervous tissue was required, the chorioallantois was the organ in which dual viral infections were established. The source of each inoculum except that of rabies was a chorioallantoic membrane previously infected with a single virus. A suspension of embryonic chick brain infected with rabies virus † was used to establish rabic infections.[8]

Development of membranal lesions following inoculations with two viruses was watched through a coverglass over an opening in the egg

* Aided by a grant from the John and Mary R. Markle Foundation.
Received for publication, September 8, 1941.
† Supplied by Dr. James R. Dawson, Jr.



Microscopically the fowlpox infection appeared to have developed normally. The laryngotracheitis infection appeared well developed but was obviously imposed upon an abnormally hyperplastic epithelium. Intranuclear inclusions of laryngotracheitis and intracytoplasmic inclusions of fowlpox were readily determined to be present in single cells (Fig. 3). Laryngotracheitis virus normally stimulates the development of large multinucleated cells, each nucleus of which contains an inclusion. The characteristic feature of the combined infections was the occurrence of large syncytia whose nuclei contained typical laryngotracheitis inclusions and whose cytoplasm was filled with large masses of Bollinger bodies (Fig. 4).

If fowlpox and laryngotracheitis viruses are inoculated simultaneously, each virus invades epithelium and produces its characteristic inclusion. Dual infection of single cells is evident but the fowlpox lesion which normally develops more slowly is dominated by the more rapidly progressive lesion of laryngotracheitis.

Fowlpox-Vaccinia Virus Infections

Seventy-two-hour fowlpox lesions were inoculated with vaccinia virus and incubated for 48 and 72 hours longer. The epithelium was hyperplastic and practically every cell contained a fowlpox inclusion. Very few Guarnieri bodies were seen and it could not be determined that the cells were doubly infected. On the other hand, 24-hour fowlpox lesions inoculated with vaccinia virus and allowed to develop 48 hours longer showed exceptionally extensive vaccinal lesions with very few fowlpox inclusions. In this case, also, dual infection of single cells could not be determined with certainty. It appears that in the first series of experiments the vaccinia virus found an unfavorable environment for growth within cells well infected with fowlpox virus. In the second series one may reasonably speculate that fowlpox virus had parasitized a large number of epithelial cells and had stimulated a hyperplastic reaction; that vaccinia virus, gaining entrance to these hyperactive cells before the slowly forming fowlpox inclusions were developed, found a favorable medium for growth and that the rapid development of Guarnieri bodies prevented almost entirely the appearance of fowlpox inclusions. Since inclusions of both these viruses are cytoplasmic, a dual infection of single cells is more difficult to recognize.

Herpes Simplex-Vaccinia Virus Infections

Membranes inoculated with mixed suspensions of herpes simplex virus and vaccinia virus were fixed after 48 hours of incubation. There was considerable necrosis and ulceration of epithelium. Microscopically, infection with each virus was evidenced by the occurrence of

shell.[9] At varying intervals infected tissues were fixed in Zenker's fluid (5 per cent acetic acid). Histological sections were stained routinely with hematoxylin and eosin.

Fowlpox-Herpes Simplex Virus Infections

Chorioallantoic membranes of 11-day embryos were inoculated with fowlpox virus and incubated at 37° C. for 48 hours. The grossly evident fowlpox lesions were then inoculated with herpes simplex virus and were allowed to develop during 72 additional hours. During the last 3 days of incubation characteristics of fowlpox infection progressed and dominated the gross appearance of the lesions.

Microscopical examinations of sections presenting 5-day fowlpox lesions and concurrent 3-day herpetic infection showed intranuclear inclusions of herpes simplex and intracytoplasmic inclusions of fowlpox within single cells (Figs. 1 and 2). As is characteristic of infection with fowlpox virus, the ectodermal epithelium was markedly hyperplastic and hypertrophic. Almost all of the cells contained typical fowlpox inclusions. In some areas the intranuclear inclusions of herpes simplex occurred in the superficial layers of hyperplastic fowlpox-infected epithelium. At other foci the herpetic infection had spread through the whole epithelial layer of cells and had invaded the chorioallantoic mesoderm. It was determined that dual infection of single epithelial cells had occurred abundantly throughout the lesion.

It was more difficult to judge how the activity of one of these viruses in a cell had modified the influence of the other virus on the same cell. The appearance of the fowlpox inclusions and the effect of that virus on the cells appeared to be entirely typical of pure fowlpox infection. This is reasonable since the fowlpox infection, which develops more slowly and with less destruction, was given a 48-hour advantage over the herpetic infection. The intranuclear herpetic inclusions appeared larger and more basophilic than usual. The herpetic infection as a whole was less extensive and decidedly less destructive to cells than commonly. Fowlpox virus usually does not invade mesodermal cells, and herpetic infection of mesoderm apparently had not facilitated its invasion by fowlpox virus. In one case metastatic foci of herpetic infection were observed in the embryonic heart, as is characteristic of herpetic infection in chick embryos, without evidence of an accompanying metastasis of fowlpox.

Fowlpox-Laryngotracheitis Virus Infections

Chorioallantoic membranes were inoculated with fowlpox virus 24 hours before laryngotracheitis virus of fowls was superimposed, and lesions were fixed 72 hours later.

Discussion

By using viruses that form recognizable intracellular inclusions, we have been able to demonstrate cytological evidence that individual cells may be invaded by, and become hosts to, two different viruses. These observations have been concerned chiefly with the parasitized cells themselves and not with the influence that one infection may have on another and concurrent infectious process in the same host. The combinations of viruses; *viz.*, fowlpox with herpes simplex, with laryngotracheitis and with vaccinia, and herpes simplex with vaccinia and with rabies, were chosen so that the occurrence of intranuclear and intracytoplasmic inclusions in one cell could be assuredly interpreted as representing dual infection of that cell.

The viruses of herpes simplex and laryngotracheitis appear to grow well within fowlpox-infected cells and these secondarily imposed infections are progressive. The same is true of herpetic infection in rabies-infected cells. Fowlpox and rabies viruses are characterized by their slow destruction of cells. On the other hand, two viruses like herpes simplex and vaccinia that very rapidly change the physiological activity of cells are by the method used only rarely found to infect one and the same cell. In no instance has it appeared that the activity of one virus within a cell rendered it more readily susceptible to invasion by another virus.

Findlay and MacCallum [11] presented as "a possible explanation of the interference shown by the neurotropic strain of yellow fever virus with the pathogenic action of the pantropic strains of yellow fever and Rift Valley fever" the idea that "when certain cells are already occupied by actively multiplying virus particles they cannot be invaded by certain other virus particles." The same interference phenomenon has been observed in experiments with certain related plant viruses.[11] Such an interference phenomenon is but rarely encountered, and investigators agree that only strains of the same virus or closely related viruses are likely to exhibit it. The mechanism of the interference is obscure.

The viruses used in this study were not related, nor does infection with any one of them protect a natural or experimental host against invasion by any other of them. These experiments show that certain different viruses may invade and multiply within single cells. It is only to this extent that the present discussion has any bearing on an interpretation of the interference phenomenon.

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characteristic inclusions. Areas in the epithelium where the infection appeared to be only vaccinal merged into other foci which were purely herpetic. It was at these margins where dual infection of single cells could be most clearly demonstrated (Fig. 5). Such doubly infected cells occurred but rarely. Although the development of an herpetic and a vaccinal inclusion within one cell did occur, it was an unusual rather than a characteristic feature of these lesions. Each virus metastasized from these complicated membranal lesions to the liver of the embryos. Vaccinal foci and herpetic foci in the liver seemed to occur independently of each other.

Rabies-Herpes Simplex Virus Infections

Twelve-day-old embryos were inoculated intracerebrally with 0.03 cc. of 1:20 suspension of chick embryo brain infected with rabies virus. After 72 hours of incubation the same embryos were given a similar inoculation of chorioallantoic tissue infected with herpes simplex virus. After another 72 hours of incubation the embryos were sacrificed for histological study. Grossly the embryos showed a marked hydrocephalus, which Dawson [10] has reported as being characteristic of rabic infection in chick embryos.

Microscopically there was extensive destruction of brain substance with somewhat less hemorrhage than is found in herpetic encephalitis. Sections stained with eosin, fuchsin and methylene blue showed intranuclear herpetic inclusions in a great many cells that also contained typical Negri bodies in their cytoplasm (Figs. 6 and 7).

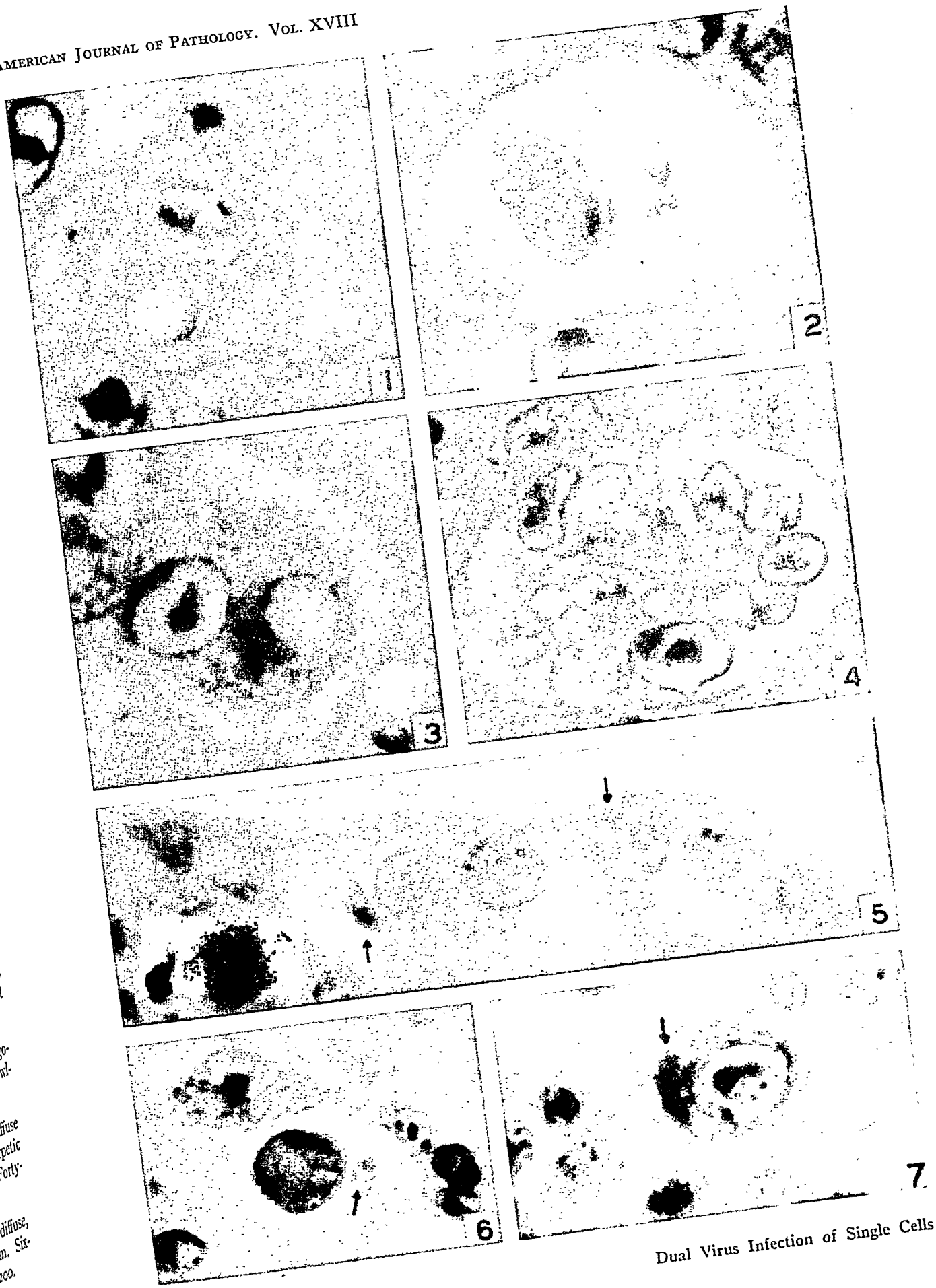
Other Attempted Dual Infections

On the assumptions that the growth of a virus within a cell depends upon the furnishing by that cell of a favorable nutritional and physiological environment for the activity of the virus, and that the growth of a virus in a cell alters the normal physiological activity of the host cell, the possibility that an already parasitized cell might be susceptible to infection by a virus to which a normal cell is resistant was considered. In these experiments, fowlpox virus was used as the primary infectious agent because it is slow to cause necrosis of individual cells.

The viruses of varicella, herpes zoster, of abortion of mares and the agent of an inclusion pneumonia in human beings have not as yet been shown to be cultivable on embryonic chick tissues. Material that might have contained one of each of these viruses was inoculated onto chorioallantoic membranes infected with fowlpox virus with the idea that a fowlpox-infected cell might offer an altered and favorable environment for the growth of one of these other viruses.

Microscopical sections showed no evidence of the growth of any of these agents except fowlpox virus itself.

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Dual Virus Infection of Single Cells

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DESCRIPTION OF PLATE

PLATE 91

FIG. 1. An ectodermal epithelial cell of chick chorioallantois showing an intranuclear inclusion of herpes simplex and a cytoplasmic inclusion of fowlpox. Five-day fowlpox lesion with concurrent 3-day herpes simplex. × 3000.

FIG. 2. A single epithelial cell showing a diffuse, granular, herpetic inclusion in the nucleus and a characteristically lobulated fowlpox inclusion in the cytoplasm at the left of the nucleus. Five-day fowlpox lesion with concurrent 3-day herpes simplex. × 3000.

FIG. 3. A single epithelial cell showing intranuclear inclusion of laryngotracheitis and cytoplasmic inclusion of fowlpox. Four-day fowlpox lesion with concurrent 3-day laryngotracheitis. × 3000.

FIG. 4. A multinucleated cell showing five nuclei containing inclusions of laryngotracheitis, with numerous fowlpox inclusions in its cytoplasm. Four-day fowlpox lesion with concurrent 3-day laryngotracheitis. × 2200.

FIG. 5. Epithelial cells showing two intranuclear herpetic inclusions of the diffuse type and two Guarnieri bodies of vaccinia. One cell infected with herpetic virus has in its cytoplasm a large vaccinal inclusion within a vacuole. Forty-eight-hour infection. × 3000.

FIG. 6. A single neuron in the brain of an embryonic chick showing a diffuse, intranuclear, herpetic inclusion and a small Negri body in its cytoplasm. Six-day rabic infection with concurrent 3-day herpetic encephalitis. × 2200.

FIG. 7. A single ganglionic nerve cell showing an intranuclear herpetic inclusion and a large Negri body in its cytoplasm. Six-day rabic infection with a concurrent 3-day herpetic encephalitis. × 2200.

DEMONSTRATION OF THE FORMATION OF RETICULIN BY SCHWANNIAN TUMOR CELLS IN VITRO *

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(From the Surgical Pathology Laboratory of the College of Physicians and Surgeons, Columbia University, and the Department of Surgery, Presbyterian Hospital, New York, N.Y.)

In a recent communication we reported the *in vitro* cultural characteristics of Schwannian cells grown from normal nerves and from neurilemomas and demonstrated to our own satisfaction that neurilemomas are tumors composed of Schwannian cells. We inferred that the collagen and reticulin fibers in neurilemomas were formed by Schwannian cells, although at that time this had not been demonstrated *in vitro*.

More recently, by variations in technical methods, we have succeeded in demonstrating the formation of reticulin by Schwannian cells from a mediastinal tumor grown *in vitro*. This confirms the inference previously expressed and offers an entirely satisfactory explanation for the presence of connective tissue fibers in tumors of purely Schwannian origin.

The patient from whom the material was obtained was a female, 49 years old, who had had first interscapular pain and later paraplegia of the lower extremities for 4 years. A dumb-bell-shaped tumor was found which compressed the cord in the region of the fourth thoracic vertebra and extended through the intervertebral foramen to the right posterior mediastinum. The portion compressing the cord was first removed and 21 months later the mediastinal prolongation was excised. Two years after the first operation, and 3 months after the second, the patient was well and free from symptoms.

The tumor was encapsulated and the second portion, removed from the mediastinum, measured 7 by 5 cm. It was solid, with a firm, fibrous peripheral zone and a large softer center which was tinted yellow because of its large lipoid content. This tumor varied in its histological appearance in different parts. In the central area it had the characteristic appearance of a neurilemoma divided into A and B tissues with an unusually large number of phagocytic foam cells filled with lipoid. The Schwannian nuclei tended to be aligned in palisades but nowhere was there a definite organoid arrangement. Here there were extremely few reticulin fibers. In the peripheral zone there were few B areas and almost no lipoid. In this region the great bulk of the tissue was composed of Schwannian cells closely packed in

* Received for publication, September 24, 1941.

Growth Characteristics in Vitro

In vitro this mediastinal neurilemoma produced a characteristic Schwannian outgrowth of the same general type as that exhibited by other nerve sheath tumors which we have studied, and by normal adult nerves (Fig. 3). As already stated, the explanted tissue was composed chiefly of A-type tissue; this constitution was confirmed in the tissue cultures, which gave rise to almost no B cells.

Within 3 days after explantation the A type of Schwann cell began to grow. This cell at first appeared in single, filamentous formation resembling some forms of neuroglia, but was often multinucleate. Later it thickened, forming a ribbon or a bundle of parallel, anastomosing filaments (Fig. 2), which still later, after a month or so of cultivation, might develop into a bundle or tuft of ribbons (Fig. 1).

Characteristic of these bundles were their lateral anastomoses. In the tissue cultures there was an almost complete absence of the compression which usually obscures the relationships between these cells *in vivo*, and this permitted illuminating observations of their growth characteristics. By continuous observation, fasciculated bundles could be seen to form in essentially the same manner as postulated by Masson from the study of a tumor nodule distended by edema. Figure 2 might serve as the source of one of his diagrams. Palisading of the nuclei often occurs *in vitro*, but usually does not last over a long period, possibly because of the freedom of movement allowed the cells by their roomy environment.

After about 10 days *in vitro* some of the cultures produced, in addition to the A cells, a semimembranous outgrowth composed of broad, flat cells resembling endothelium which were usually to be found on the surface of the clot, as contrasted with the Schwannian cells which characteristically push their way through the clot. When treated with silver nitrate this semimembranous outgrowth was shown to form a mosaic of cells with blackened cement borders—characteristic of endothelium but not of Schwannian cells. It was therefore supposed that such cells were derived from the blood vessels of the tumor.

A 5-year study of human and animal, fetal and adult, normal and abnormal nerve sheath tissue *in vitro* has convinced us that Schwannian cells can be distinguished positively from fibroblasts in this medium, on the basis of growth characteristics and pattern, and on general morphology and physiology. We have no hesitation, therefore, in stating that some cultures, and many areas in all the cultures, were entirely free from cells of fibroblastic origin. Only such areas will be considered in the following discussion of fiber formation by Schwannian cells *in vitro*.

masses and accompanied by many collagen and reticulin fibers. Some of these areas closely approached the appearance of neurofibroma. Chloral hydrate fixation and Cajal impregnation failed to show any neurofibrils. We were of the opinion that the tumor should be classified as a neurilemoma but it demonstrated what we have observed before—that neurofibroma and neurilemoma are in fact only different growth manifestations stemming from a single basic origin, namely, the Schwannian syncytium. Blood vessels consisting of endothelial lining and a rather thick collagenous sheath were scattered irregularly throughout the tumor.

The tissue used for explantation came from the periphery and included a larger proportion of the solid fibrous zone and a smaller amount of the lipoid-containing central area of characteristic neurilemomatous aspect.

Methods

The Maximow double-coverslip method of handling a hanging, or rather, a lying drop was used exclusively in the tissue cultures. These were explanted in a medium composed of 2/10 chicken plasma, 5/10 human placental serum, 1/10 chicken embryo extract and 2/10 serum ultra-filtrate, prepared by Dr. H. S. Simms from beef blood. The use of this medium reduced to a considerable extent the liquefaction of the clot which usually accompanies the growth of these tumors *in vitro;* so that it was generally possible to keep the cultures in their original situation on the coverslip without transferal throughout the period of cultivation. At intervals of 2 to 4 days they were washed in a buffered saline solution (Simms and Sanders) and the liquid components of the medium were subsequently renewed. Occasionally they were patched with fresh chicken plasma. For the washing it was found convenient to set the cultures vertically in small, covered Coplin jars designed to hold coverslips.*

At intervals during the 57-day period of cultivation, cultures were fixed in Zenker's or Helly's fluids and stained with Delafield's or phosphotungstic acid hematoxylin or with fuchsin-ponceau and aniline blue. Zenker's fluid was found to be the most favorable fixative for preceding silver impregnation for reticulin. For this the Bielschowsky method was used, as adapted for tissue cultures by McKinney from Foot and Mènard's modification. In all these procedures, coverslip Coplin jars were employed and the cultures handled in an upright position, which greatly facilitates drainage. The silver impregnations were counterstained lightly with toluidine blue, in 0.04 per cent aqueous solution.

* These jars, which are exceedingly useful in the various operations of tissue culture, are purveyed by the Arthur H. Thomas Co. of Philadelphia.

fibroblastic derivation. The process of fiber production by Schwannian cells differs in certain respects from fiber production by cells of mesoblastic origin.

NOTE: We are greatly indebted to Irene Bokoff for technical assistance in the handling of the tissue cultures, and to Walter I. O'Neill for the photography.

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Reticulin Formation by Schwannian Cells

Reticulin formation in these cultures was slow, sparse and sporadic. The reticulin appeared first as a row of fine rods and granules which later coalesced to form long, single fibers running parallel to the long axis of the Schwann cell. The fiber always originated in close relationship to the cell, often appearing to lie in contact with the long-drawn-out cytoplasmic portion and making a curved detour around the lateral surface of the nucleus. Growth and migration of cell or bundle sometimes left these fibers behind without connection with any cell, but they did not so originate. They always appeared to develop singly, not as the mat of fibers described by McKinney in the mammalian lymph node, or as bundles of fibers described by Stearns in rabbit connective tissue.

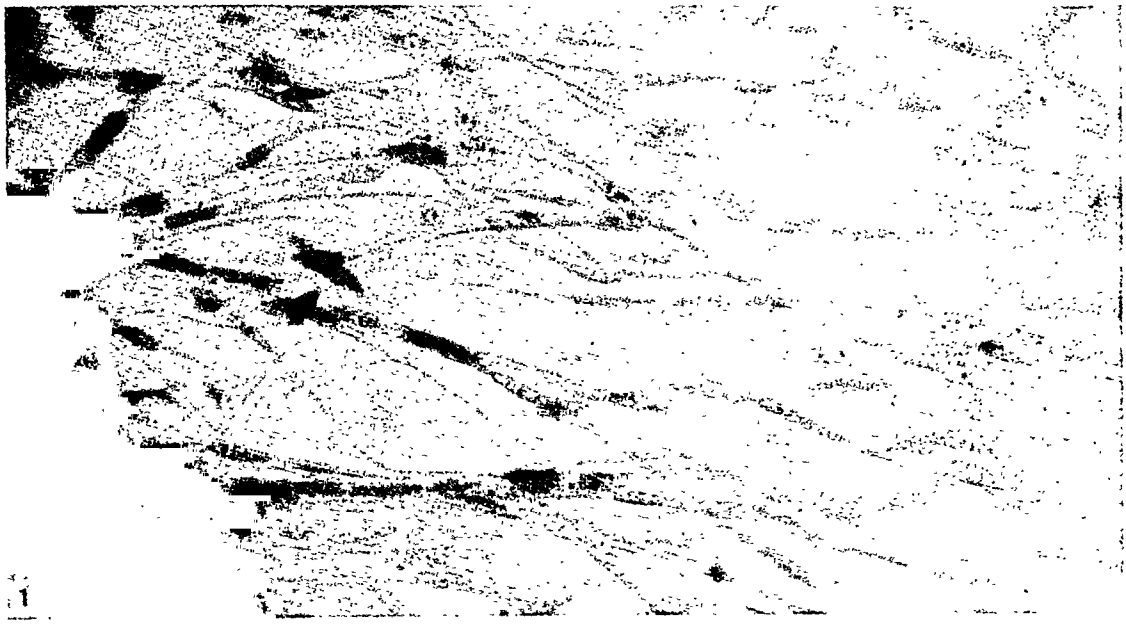
In general, the reticulin fibers shown by silver impregnation were not in contact with fibers in the explant, but occupied a zone adjacent to the explant. Fibers which stained with aniline blue were always in contact with old fibers in a zone of dense outgrowth, so that it was difficult to be certain where the new growth of fibers began.

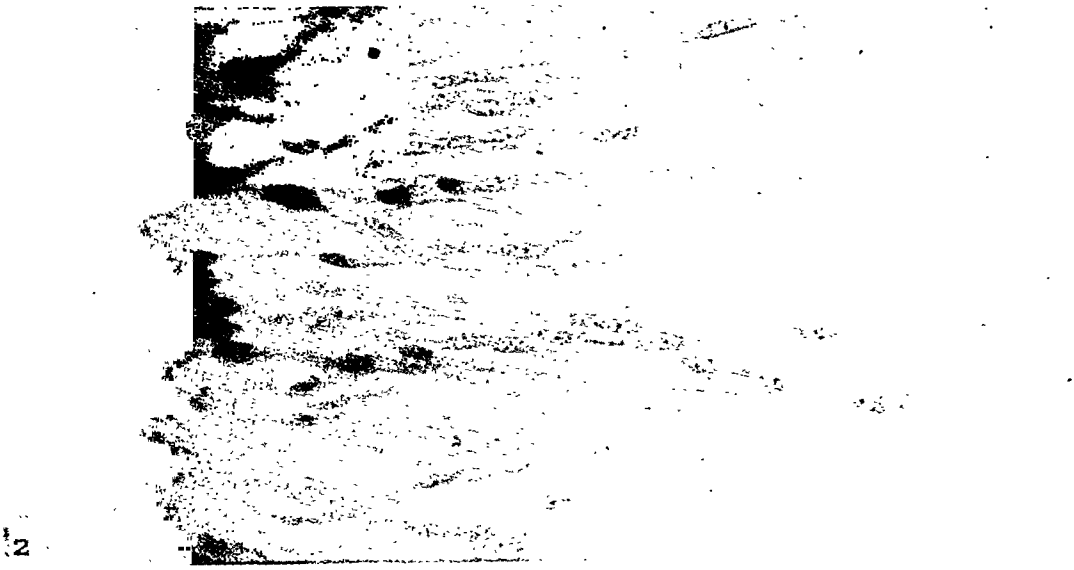
The first argyrophil fibers were observed in a 13-day culture. They were present in some older cultures and not in others. There was no orderly progress in fiber formation among the cultures as a whole. The speed and intensity of the process varied from culture to culture, as it appears to do in various areas of a tumor. By comparison, cultures of the stroma of an adenomatous human parathyroid gland, cultivated in the same medium under similar conditions, laid down reticulin fibers very much more densely, regularly and rapidly. Such fibers appeared first as criss-cross bundles in contact with the explant (Fig. 6).

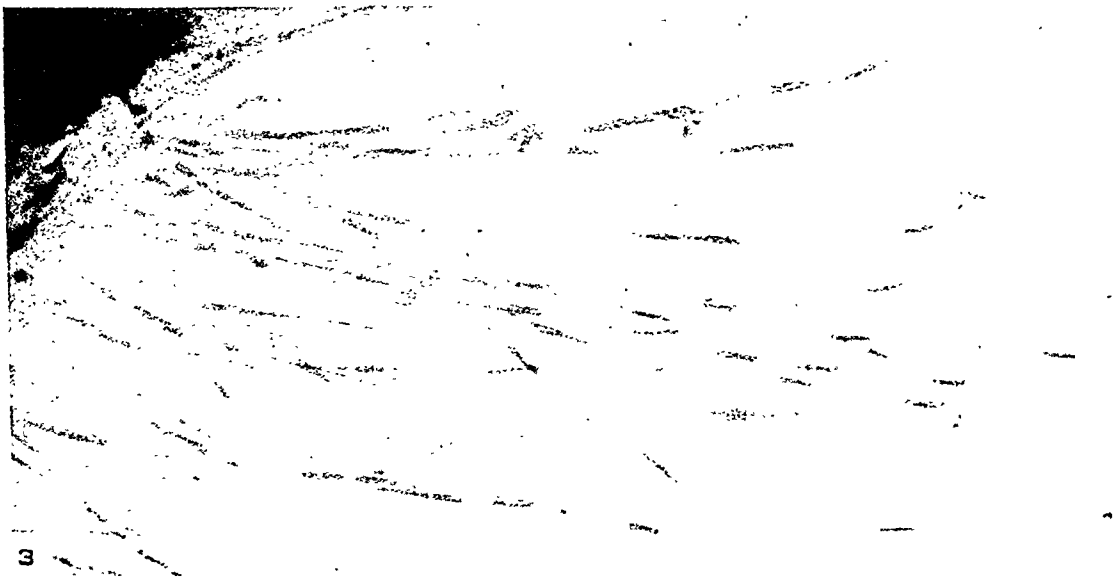
In the neurilemoma cultures, reticulin was rarely seen in connection with macrophages or structures believed to be derived from blood vessels present in the explants. Great care was taken to exclude all such areas from consideration in the material used for the foregoing description of reticulin formation around Schwannian cells, and only cells and bundles which could be positively identified as Schwannian were included. Figures 4 and 5, showing reticulin, represent cell formations essentially similar to those of Figures 2 and 1, in which the cell structure and arrangement are brought out with hematoxylin staining.

Summary

Reticulin fibers have been shown to arise *de novo* in connection with Schwannian cells growing out from a mediastinal neurilemoma cultivated *in vitro*. The areas under consideration were devoid of cells of







Murray and Stout Formation of Reticulin by Schwannian Tumor Cells

DESCRIPTION OF PLATES

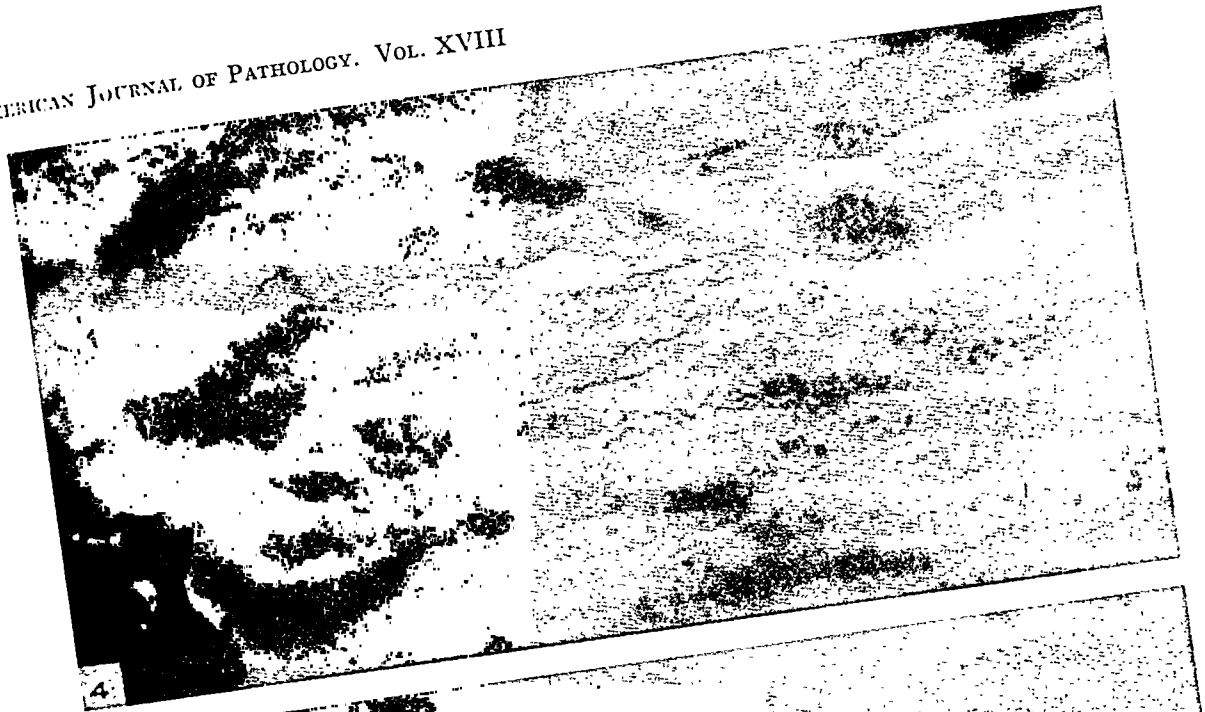
PLATE 92

FIG. 1. Neurilemoma from mediastinum (no. 77393) after 24 days *in vitro*, showing Masson's fasciculated bundles of Schwann cells. Zenker's fluid, Delafield's hematoxylin stain.

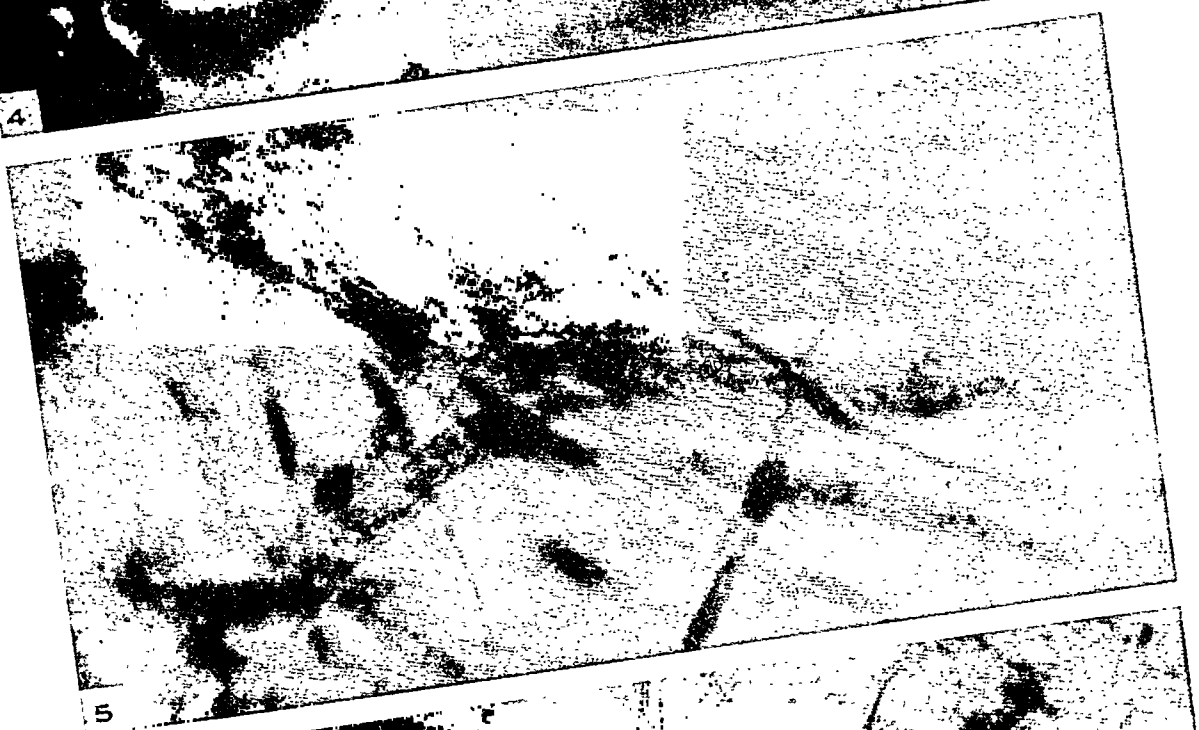
FIG. 2. Neurilemoma from mediastinum, after 15 days *in vitro*, showing "aneuritic bundles" of Schwann cells, expanded in one plane. Helly's fluid, Delafield's hematoxylin stain.

FIG. 3. Schwannian outgrowth from small medullated fibers in normal human adult celiac nerve, after 24 days *in vitro*. Carnoy's fluid, Delafield's hematoxylin stain.

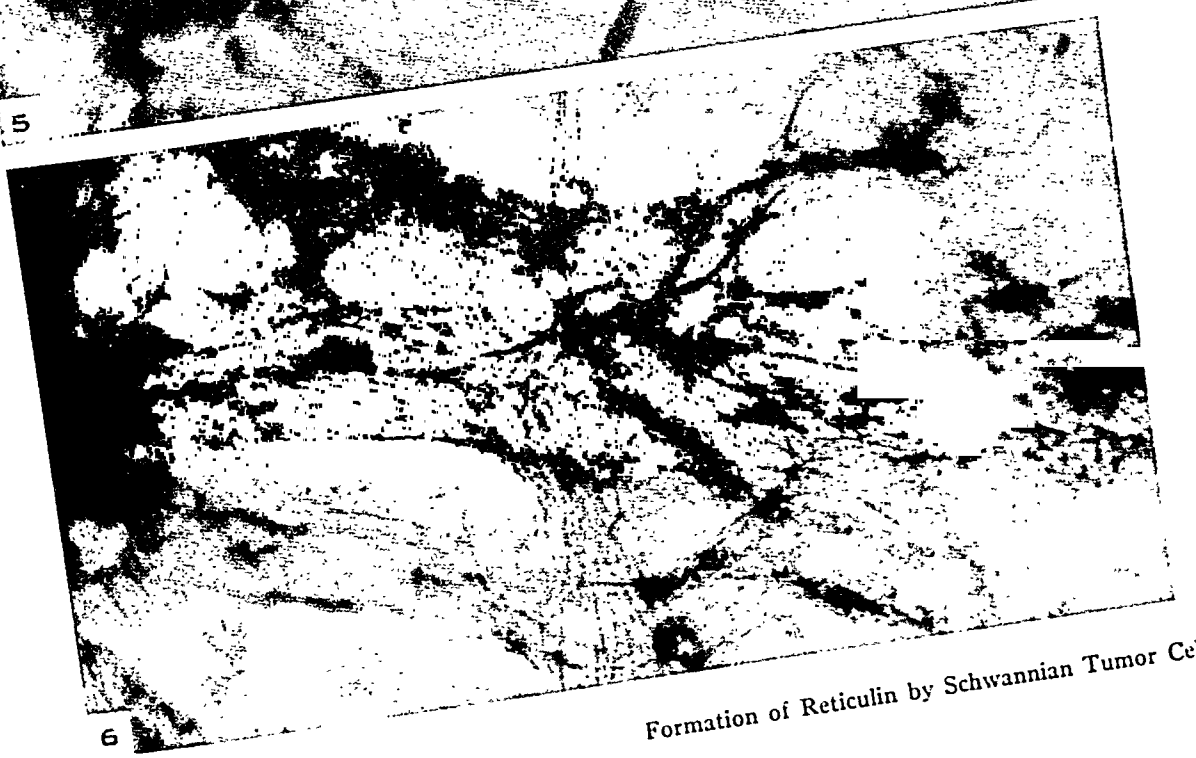
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4



5



6

Formation of Reticulin by Schwannian Tumor Cells

PLATE 93

(All three photomicrographs were taken at the same magnification, with high dry objective lens.)

FIG. 4. Neurilemoma from mediastinum (no. 77393) after 24 days *in vitro*, showing longitudinal reticulin fibers formed between Schwann cells. Zenker's fluid, Foot's silver impregnation.

FIG. 5. Similar to Figure 4, showing longitudinal fibers in a bundle of Schwann cells like those of Figure 1.

FIG. 6. Parathyroid adenoma (no. 76598) after 20 days *in vitro*, showing matted reticulin fibers formed among fibroblasts from the stroma of the tumor. Zenker's fluid, Foot's silver impregnation.

AN ANATOMICAL STUDY OF THE CLOSURE OF THE DUCTUS ARTERIOSUS *

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The successful surgical ligation of a patent ductus arteriosus [1] has aroused new interest in this structure. This report concerns an anatomical study of a series of ductus arteriosi in an attempt to determine the mechanism of closure.

The method of closure of the ductus arteriosus has long been a disputed point. The approach to this problem resolves itself into the factors concerned with the immediate physiological closure occurring shortly after birth and into the subsequent anatomical obliteration of the lumen.

The early concept that thrombosis was the immediate cause of closure has been discarded. Thrombi have been demonstrated rarely in the ductus arteriosi of infants [2,3] but are considered abnormal. Rauchfuss [4] found the ductus arteriosus thrombosed only four times in 1,400 infants. The ingenious theory of Strassmann [5] postulated that immediate closure of the ductus arteriosus is effected by means of a valvelike intimal fold situated at its aortic orifice. After birth the increased blood pressure in the aorta closes the valve, preventing reflux into the pulmonary artery. It has been well established that the ductus arteriosus enters the aorta at an acute angle and that the entrance into the pulmonary artery is less acute. Roeder [6] found that in newborn infants the angle of entrance of the ductus arteriosus into the aorta is always an acute angle of about 33 degrees. This has been confirmed by Gräper.[7] Strassmann, and later Fromberg,[8] carried out a number of injection experiments in infants which purported to show that the ductus arteriosus could not be injected from the aortic side with greatly elevated pressure, although the injection from the pulmonary side allowed easy passage of fluid through the ductus arteriosus into the aorta. This work was regarded as proof of the effectiveness of the Strassmann valve. Fromberg pointed out the similarity of the entrance of the ductus arteriosus into the aorta with the entrance of the ureters into the bladder. The results of injection technic and the effectiveness of the Strassmann valve have been disputed by Gräper, Linzenmeier [9] and others. Thus Gräper believed the elevated intimal fold at the angulated junction of the ductus arteriosus with the aorta too small to be effective.

* Received for publication, August 21, 1941.



Confusion exists as to the histological structure of the ductus arteriosus in the newborn infant and in the subsequent anatomical changes leading to closure of the lumen. Rokitansky [16] thought there was no difference in the histological structure between the ductus arteriosus and the aorta. Subsequent investigators (Gräper,[7] Linzenmeier,[9] Melka,[11] von Hayek,[12] Swensson [13] and many others) have shown this to be incorrect. There is general agreement that the ductus arteriosus of the newborn is looser in structure than the great vessels, that it has a well defined internal elastic lamina, has less elastic tissue than the great vessels, and that it has no external elastic lamina. There are conflicting views as well. Thus the direction of the muscle fibers in the media of the ductus arteriosus was described by von Hayek as running in spiral lines. Gräper described an inner longitudinal layer of muscle fibers and an outer circular layer with interspersed longitudinal bundles. Melka found an inner and outer longitudinal layer and a central circular layer.

Swensson [13] made a careful histological study of a series of ductus arteriosi taken from human fetuses ranging from 3.5 to 47 cm. in length. Even in a 4.5 cm. fetus he observed that the ductus arteriosus had less elastic tissue and was looser in structure than the aorta and pulmonary arteries. In a 10 cm. fetus there was a well defined internal elastic lamina. In this fetus there was abundant collagen in the media of the pulmonary artery and aorta, yet there were only a few collagen strands in the media of the ductus arteriosus and these lay in the outer portion of the media. In a 23 cm. fetus he noted intimal pillows, *i.e.*, eccentrically thickened intimal mounds composed of smooth muscle and elastic tissue. Beneath these mounds the internal elastic lamina was frequently fragmented and split. The pillows persisted in progressively older fetuses. From the 23 cm. fetus to the full-term infant there was no significant change in the structure of the ductus arteriosus. Gräper [7] found that the ductus arteriosus of the newborn infant possesses thick intimal mounds. He pointed out the presence of holes in the loose inner portion of the media. These holes are situated most frequently near the bases of the intimal mounds and often contain precipitated plasma and cellular elements of the blood. Gräper showed that with progressing age there are changes in the structure of the ductus arteriosus. The intimal pillows become thicker, with resulting narrowing of the lumen. The media becomes thicker and more compact and the holes in the media vanish. As early as 8 days after birth there is evidence of ischemic degeneration in the inner portion of the media with subsequent progression to include the intima and its mounds. There is a relative increase in elastic tissue due to degeneration of other structures. The lumen is visible for a period

Schanz [10] believed immediate closure of the ductus arteriosus resulted from stretching and knuckling of this structure produced by a change in position of the thoracic organs effective with the onset of respiration. Linzenmeier [9] made careful comparative anatomical studies of stillborn fetuses and infants who had died after breathing and believed he could show a definite change in position of the heart and great vessels after respiration with resulting knuckling of the ductus arteriosus. In addition, he injected the ductus arteriosus of stillborn infants, then inflated the lungs and obtained moulds which demonstrated a knuckling of the ductus arteriosus following insufflation. This work was criticized by Gräper,[7] who made similar studies and concluded that while there was a knuckling of this structure with full inspiration, the knuckling was much less pronounced with expiration and therefore could not be the responsible factor in immediate closure. A mechanism similar to that postulated by Schanz was that of Melka,[11] who presumed that at birth the pulmonary artery and aorta become distended with blood and compress the ductus arteriosus, producing immediate closure.

On the basis of the histological structure of the wall of the ductus arteriosus, effective closure by active muscular contraction has been a popular concept, supported by Gräper,[7] von Hayek,[12] Swensson [13] and many others. Thus von Hayek was impressed by the similarity in structure of the muscle fibers of the umbilical artery and of the ductus arteriosus. Gräper was of the opinion that the same stimulus which leads to contracture of the umbilical artery also leads to contracture of the ductus arteriosus. Recently new impetus has been given to this theory by the work of Barclay, Barcroft, Barron and Franklin.[14,15] These workers used lamb fetuses delivered by cesarean section, leaving the maternal circulation intact and preventing respiration by holding a rubber bag containing amniotic fluid over the snouts of the fetuses. Radio-opaque dye was injected into the external jugular veins or the umbilical arteries of the fetuses and the outlines of the heart and great vessels were visualized and recorded with motion pictures. It was found that the ductus arteriosus of these fetuses became closed soon after birth (in one case, in 5 minutes) and that this closure occurred even in the absence of respiration and without interruption of the maternal circulation. In several instances they were able to force the dye into the femoral arteries of moribund fetuses in which the maternal circulation had been interrupted. The dye extended in a retrograde fashion into the aorta, ductus arteriosus and coronary arteries. From their studies they concluded that the pulmonary end of the ductus arteriosus is the first portion to close.

taken. The usual procedure was to take transverse sections at the aortic and pulmonic ends of the structure and also a transverse section of the central portion. Frequently, however, longitudinal sections of the pulmonic and aortic ends of the ductus arteriosus and transverse sections of the central portion were taken. The longitudinal sections included portions of the adjacent pulmonary artery and aorta. Sections were stained with phloxine and methylene blue and with Verhoeff's elastic tissue stain, using the van Gieson counterstain.

Gross Findings

All except 5 in this series of 71 ductus arteriosi were cylindrical in type. The acute angulation at the junction with the aorta was a constant occurrence. The ductus arteriosus varied from 0.5 to 1.3 cm. in length and from 0.1 to 0.8 cm. in diameter, after fixation. The average measurements in this series were 0.8 cm. in length and 0.3 cm. in width. Anatomical closure usually occurred by the end of the third week of life. An occasional vessel was found to be patent as late as the end of the second year. Three ductus arteriosi of the window type were observed in which the aortic and pulmonic orifices were closely approximated without any demonstrable intervening structure which could be identified as a vessel. All specimens of the window type were widely patent. These were obtained from infants of 3 days, 4 days and 3½ months of age. In addition there were two adults with a ductus arteriosus of the cylindrical type closed only at the pulmonic end.

Histological Findings

The ductus arteriosus presents a histological appearance similar to that of any systemic artery of equal size, with an intima, well defined internal lamina, media and adventitia. By intima is designated the portion of vessel wall lying internal to the internal elastic lamina. The intima is peculiar in that there are mounds which project into the lumen. These mounds, which contain fine elastic fibers, smooth muscle and later collagen, are an integral part of the mechanism of closure. They vary in number, width and length in a given cross section and in different portions of the same vessel. The media, in contrast to that of the aorta and pulmonary artery, is loose in structure and contains fine, wavy elastic fibers rather than coarse longitudinal bundles. No external elastic lamina is present in the ductus arteriosus. The adventitia, poorly delimited from the outer portion of the media, contains collagen, elastic fibers, smooth muscle and small blood vessels.

The peculiar elastic structure of the ductus arteriosus in contrast to that of the aorta and pulmonary artery is effectively shown in longi-

of 1½ years but is occluded at each end. Throughout life the characteristics of a vessel are maintained. Melka [11] explained the closure as follows: Obliteration is a proliferative process, which he observed beginning as early as in a 5.5 cm. fetus. The internal elastic membrane proliferates in an asymmetrical fashion, forming mounds which project into the lumen. Gradually collagen grows from the mounds out into the lumen, effecting complete closure. Weizmann [17] stated that in old age, calcification, cartilage and fat frequently appear in the course of the ductus arteriosus. Benninghoff [18] observed that in the proliferating process, elastic fibers and connective tissue participate. In the scar of the ductus arteriosus in old age there is frequently calcium deposition. A stellate-shaped lumen most frequently remains in the central portion. Finally the structure is converted into a connective tissue strand in which elastic fibers and smooth muscle persist.

The time required for complete closure of the ductus arteriosus is variable. Mönckeberg [19] stated that any ductus arteriosus which remains open longer than 3 months should be regarded as patent, but added that occasional cases occur in which closure is effected in the second and third year. Christie,[20] in a series of 558 autopsied infants, found that at 2 weeks, 64 per cent of the ductus arteriosi were still patent; at 6 weeks, 20 per cent; at 12 weeks, only 5 per cent, and by the end of a year, only 1.2 per cent were still patent. Vierordt [21] considered the ductus arteriosus obliterated at the end of 20 days. Théremin [22] found that the ductus arteriosus was open in 23 per cent of 144 infants varying from 1½ to 4 months of age. Théremin observed that the obliterative process begins in the middle of the ductus arteriosus, progresses next to the pulmonic end and finally to the aortic end. According to Rokitansky,[16] the pulmonic end narrows first.

Gerhardt [23] classified patent ductus arteriosus in adults into four types: (1) cylindrical type, (2) funnel-shaped type with the dilated portion at the aortic orifice, (3) window type with little or no separation between the aortic and pulmonary orifices, and (4) an aneurysmal dilatation of any portion. In addition there is a fifth type in which there is incomplete patency with closure occurring only at the pulmonic end. This type has been described by Hebb,[24] Mönckeberg [19] and Altschule.[25]

Material and Methods

Our material is composed of a series of 71 ductus arteriosi representing a range from 28 cm. fetuses to adults 80 years of age. The specimens were fixed in Zenker's solution. The loose connective tissue was then removed from the vessel wall, measurements made and sections

organizing thrombus. The intimal mounds were variable in size but seemed slightly larger than in younger specimens and in three instances contained small amounts of collagen. Two of these ductus arteriosi were striking in that there were caps of cellular connective tissue projecting into the lumen from the surfaces of the mounds. These caps, which were subendothelial, apparently arise from an outgrowth of the connective tissue of the intimal mounds and can be differentiated readily from the intimal mounds because of the absence of elastic fibers in the caps. No changes in the structure of the media were observed.

The ductus arteriosi of six infants varying from 13 to 21 days of age showed progressive changes toward obliteration of the lumen. The lumen of one was occluded by an organizing thrombus. In this same specimen there was hemorrhage into the intima with extension into the media. In an infant, 21 days old, the lumen of the ductus arteriosus was occluded by vascular connective tissue, suggesting fusion of expanding connective tissue caps, although a revascularized organized thrombus could not be excluded. In three cases the lumina were markedly narrow and in one the lumen was widely patent. The intimal mounds present in this group were rich in elastic tissue and contained an increasing amount of collagen. The internal elastic lamina was maintained except at the mounds where it was fragmented. The media was more cellular, slightly thicker and more compact with an increase in elastic tissue and collagen.

There was anatomical closure of four ductus arteriosi taken from infants varying from 22 to 30 days of age. In several, a slitlike orifice persisted in the central portion while one or both ends were occluded by expansion of the intimal mounds. In two the central portion was occluded by organizing thrombi. In all the intimal mounds were large, containing variable amounts of collagen and elastic tissue. Degenerative changes, as evidenced by acellular pink-staining material rich in elastic fibers, were present in the intimal mounds of two specimens. The media was similar to that of the previous group.

From the period of 1 to 3 months of age, six ductus arteriosi were studied. In five the lumina were occluded. This occlusion was effected largely by a proliferation of the intimal mounds with or without overlying caps of loose connective tissue. In one instance the lumen was occluded by an organized thrombus, while another had an unorganized thrombus in the lumen with hemorrhage into an intimal mound. In an infant, 3 months old, the lumen of the ductus arteriosus was patent but narrowed to a small slit throughout its course. The intimal mounds in all were increasingly rich in elastic tissue and contained variable, often

tudinal sections of this vessel and the adjacent great vessels. The elastic fibers of the aorta and pulmonary artery condense into a coarse elastic band at the orifice of the ductus arteriosus. The major portion of the elastic fibers traverses obliquely to the adventitia at each end of the ductus arteriosus and terminates. A small portion forms the internal elastic lamina of the ductus arteriosus and the loose, fine meshwork of elastic fibers of the media.

Two 28 cm. fetuses were sources of the earliest specimens in this series. In these the lumina were widely patent. The thick internal elastic lamina was subendothelial except in several areas where low mounds projected slightly into the lumen. These mounds, which were composed of smooth muscle and moderate numbers of fine, wavy elastic fibers, appeared to arise from a splitting and reduplication of the underlying internal elastic lamina. The media was loose, thin, and consisted of smooth muscle and fine, wavy elastic fibers. Just beneath the internal elastic lamina the muscle fibers of the media had an oblique or radial arrangement which was most marked beneath the intimal mounds. Longitudinal muscle fibers flanked the radial fibers. The muscle fibers in the outer portion of the media were circular with a few interspersed longitudinal fibers. There was no collagen in the intima and the small amount which was present in the media lay near the adventitial border.

The ductus arteriosi of seven fetuses varying from 33 to 45 cm. in length showed slight changes from the 28 cm. fetuses. The lumina remained widely patent. The intimal mounds, which continued to be composed of elastic fibers and smooth muscle, showed a definite but variable degree of enlargement in respect to their height and width. Beneath the intimal mounds the tendency to fragmentation of the internal elastic lamina was more marked. The media showed no structural change from the previous specimens.

Five ductus arteriosi taken from full-term infants, ranging from a stillborn infant to those living 3 days, showed very little change. The intimal mounds were slightly larger, contained a moderate amount of elastic tissue and in several instances contained also a slight amount of collagen. The media remained loose and thin, with the muscle fibers coursing in the fashion previously described. In the midportion of the media of several of these specimens there were cellular whorls which were composed predominantly of concentric layers of smooth muscle fibers.

Four specimens obtained from infants living 4 days showed minor changes from those previously mentioned and in addition showed variations among themselves. The lumina of three were widely patent, whereas the lumen of the fourth was completely occluded by an early

by a mass of dense elastic tissue, collagen, hyaline material and a small amount of smooth muscle which lay toward the outer portion of the vessel wall. In longitudinal sections the intima of the pulmonary artery and aorta became thickened over the obliterated ends of the ductus arteriosus. This thickening, although less pronounced, was also present in younger age groups. It is at this site that atheromatous plaques are a common occurrence in the aortas of adults, even in the absence of marked atheromatous lesions elsewhere in the aorta.

Several unusual histological changes were observed in this whole series of ductus arteriosi. In the three patent ductus of the window type, there was, at the site of junction of the pulmonary artery and aorta, a slight thickening of the adjoining intimas, and a contrasting looseness of the media, with elastic tissue streaming from each major vessel toward the adventitial surface. The thickened intima contained elastic tissue, smooth muscle and, in one instance, collagen. There was no internal elastic lamina at the site of junction.

In four instances dissecting aneurysms were observed in the midportions of the cylindrical ductus arteriosus. Two specimens, which were from twins, 45 cm. in length and living 12 and 14 hours respectively, showed fresh hemorrhage in the inner and midportion of the media with elevation of the intima. The pulmonic and aortic ends were not involved. Vascular structure was otherwise similar to that previously described in this age group. One specimen from a full-term infant surviving for 12 hours showed mucinous degeneration with cyst formation in the midportion and outer portion of the media with recent hemorrhage involving half the circumference of the vessel. In an infant, 15 days old, the ductus arteriosus showed a hemorrhage extending directly from the lumen into the outer half of the media. In none of these four was there any evidence of rupture into the adventitia or any evidence of extension into the adjoining great vessels.

Summary and Conclusions

From a series of ductus arteriosi obtained from 71 autopsies, giving a range from 28 cm. fetuses to individuals 80 years of age, the mechanism of closure was studied. In a 28 cm. fetus the ductus arteriosus has a well defined internal elastic lamina which is subendothelial, except in several areas where there are low intimal mounds projecting into the lumen, rendering it eccentric. The mounds, which are composed of smooth muscle and fine elastic fibers, appear to arise from the internal elastic lamina which has a tendency to fragment beneath them. The media is loose in structure and is composed of fine, wavy elastic fibers and smooth muscle fibers. This affords a striking contrast

large, amounts of collagen. Smooth muscle fibers became less conspicuous in the intimal mounds. No degenerative changes were present in the specimens in this group. The internal elastic lamina was frequently obscured by the abundance of elastic fibers in the intima and media. The media was dense, thick and rich in elastic fibers. With the increased thickness of the media the muscle fibers were almost entirely circular, with disappearance of the abundant longitudinal and radial fibers which were observed in earlier groups.

The ductus arteriosi of ten infants from 3½ months to 1 year of age were examined. In every instance the lumen was occluded. The obliterated lumen contained a sparsely cellular tissue rich in collagen and elastic fibers. At 5 months of age degenerative changes were observed in the intima. By 7 months of age the lumen of the ductus arteriosus was occluded by acellular hyalinized material rich in elastic tissue. There was a variable degree of vascularization in the obliterated lumina. The ductus arteriosus of one infant, 6 months old, showed degenerative changes below the internal elastic lamina or in the inner portion of the media. The media otherwise showed no significant change from the previous group.

In a series of eight specimens taken from children varying from 1 to 7 years of age the lumina were occluded in five. In one child, 14 months old, and in another, 2 years old, the lumina of the ductus arteriosi were patent but persisted only as narrow slits. The lumen of a specimen obtained from a child of 4 years contained an unorganized thrombus. Degenerative changes in the vessel wall were more marked than previously. The media was dense and rich in collagen and elastic tissue. In this group, as in some of the older groups, there was occasionally a dense, thick elastic membrane lining a slitlike lumen. Further from the central axis there was often a second incomplete elastic lamina which probably represented the remaining fragments of the original internal elastic lamina.

Sixteen ductus arteriosi were obtained from adults varying from 16 to 80 years of age. The central portion of a specimen from a young adult, 16 years old, was occluded by an organizing thrombus while the pulmonic and aortic portions were completely obliterated. In two adults, 18 and 20 years old, small slitlike lumina persisted throughout the course of the ductus. The remaining specimens were closed completely, or only at the pulmonic end as observed in two adults of 20 and 48 years of age. Degenerative changes were more marked, especially in elderly people where calcification, bone formation and cartilage were occasionally observed. The borderline between intima and media became lost and the entire structure was usually represented

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to the aorta and pulmonary artery where the media is compact and composed of laminated bundles of dense elastic fibers. In the ductus arteriosi of full-term infants the only variation from this pattern lies in the slight increase in size and number of the intimal mounds.

Anatomical closure is effected largely by an increase in size and perhaps in number of these intimal mounds, which gradually become infiltrated with collagen. Frequently, subendothelial caps of loose connective tissue grow from the surfaces of the intimal mounds and assist in the obliterative process.

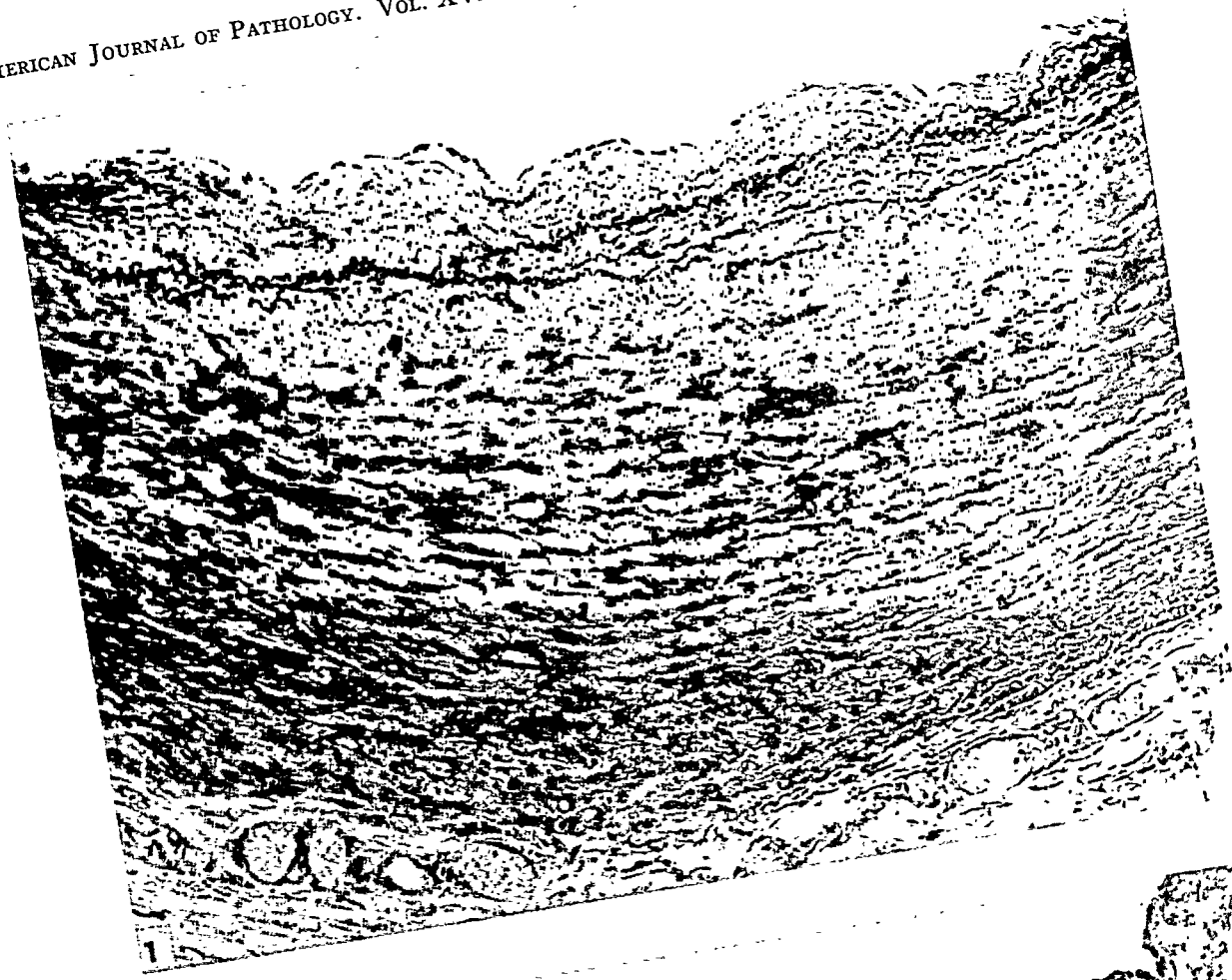
During, and subsequent to, the process of obliteration of the lumen the intima gradually becomes more rich in elastic tissue. The media becomes denser, thicker, and richer in collagen and elastic tissue. The internal elastic lamina usually becomes obscured by the dense elastic tissue of the intima and media. Degenerative changes appear in the vessel wall and in the obliterated lumen as early as 5 months of age. Anatomical closure of the lumen of at least a portion of the course of the vessel is usually effected by the third or fourth week of life. A small, slitlike, microscopical lumen may persist for several months or occasionally longer. It appears that the central portion of the ductus arteriosus remains histologically patent longer than the aortic and pulmonic ends. In a few instances the pulmonic end is the first portion to become occluded. Thrombosis may occur in the lumen at any time from 4 to 30 days of age but has been observed as late as 4 years of age in this series. This is not a constant finding. In older age groups the ductus arteriosus consists of a dense mass of collagen and elastic tissue with only a few remaining muscle fibers. Hyalinization, calcification and even cartilage formation occur in this group.

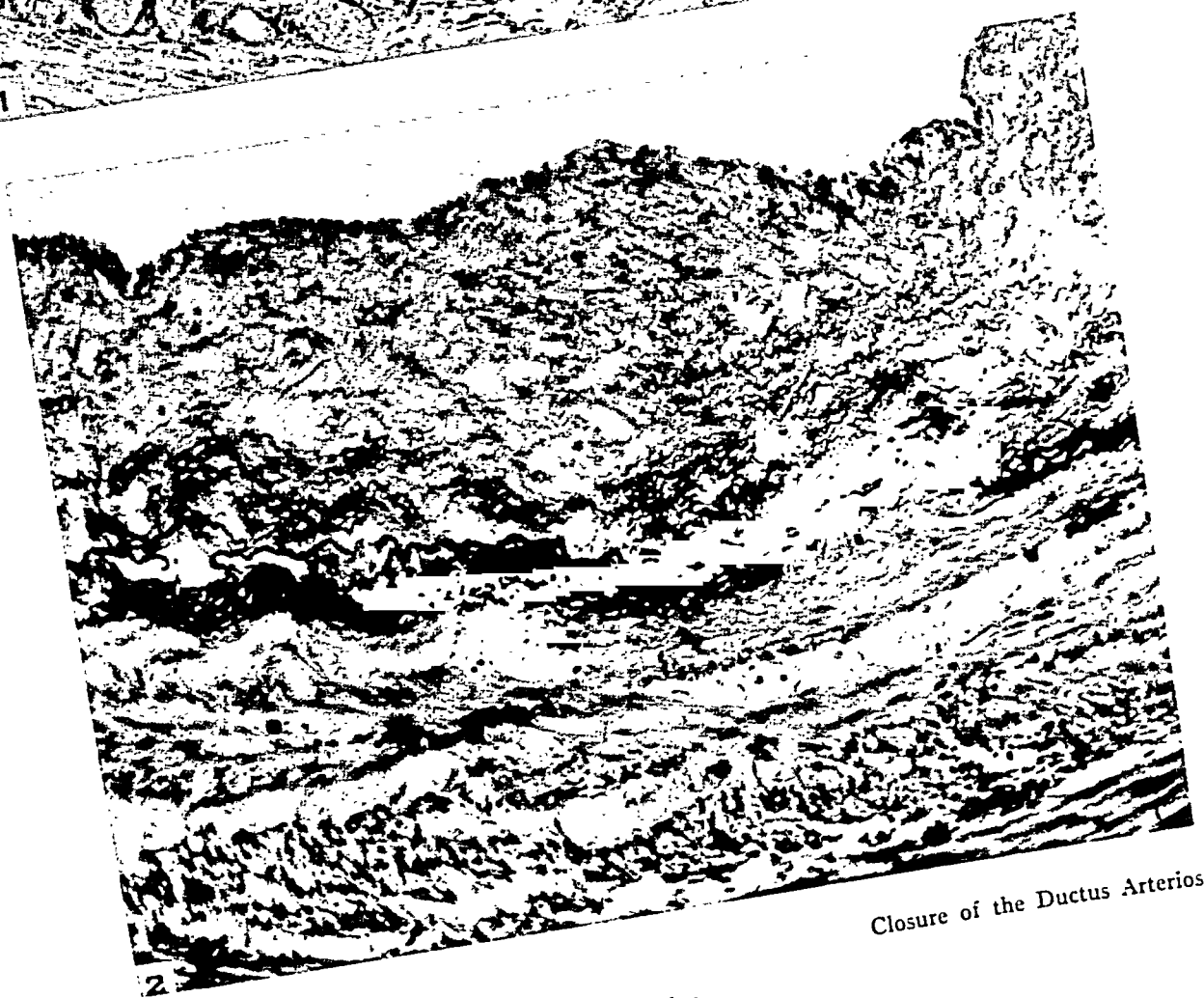
It is apparent from this series of ductus arteriosi that many variations in histological structure from this described pattern may occur. These variations may in part account for the seemingly contradictory observations of other investigators.

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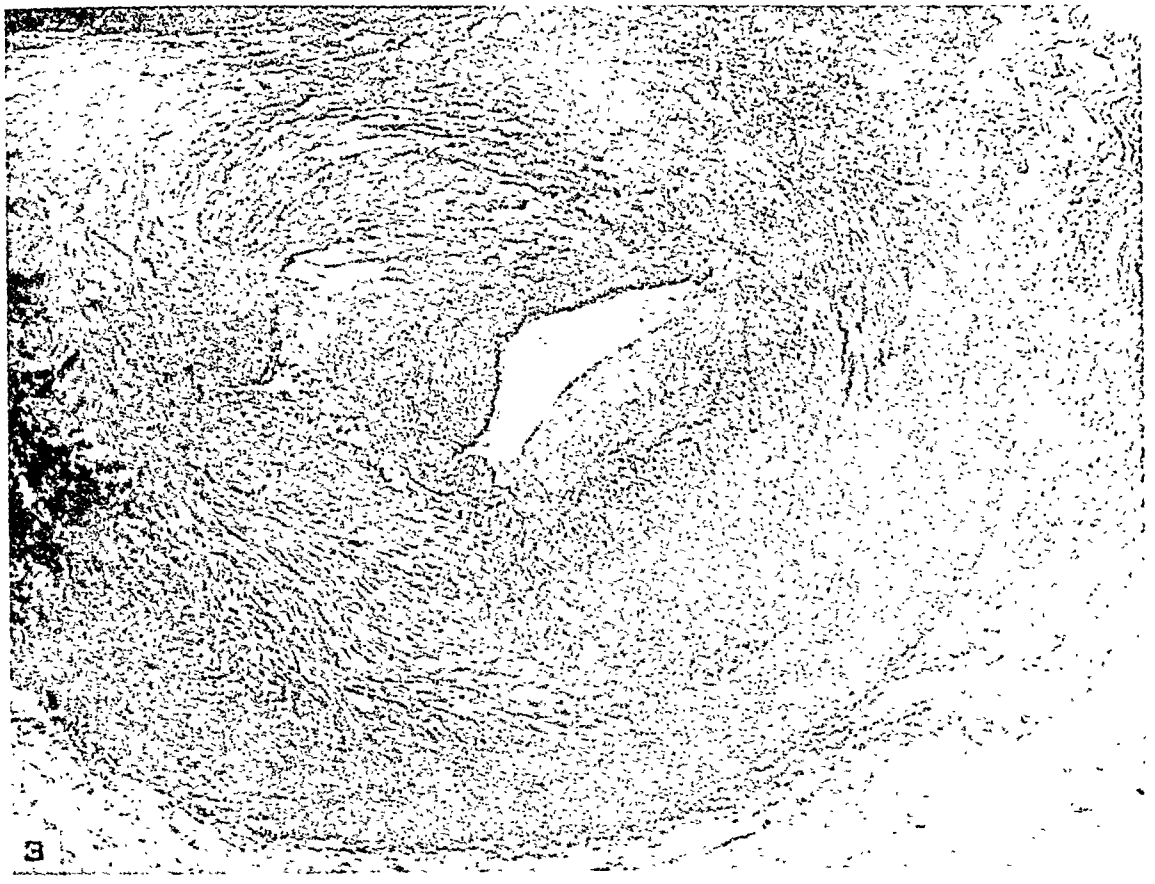
Closure of the Ductus Arteriosus

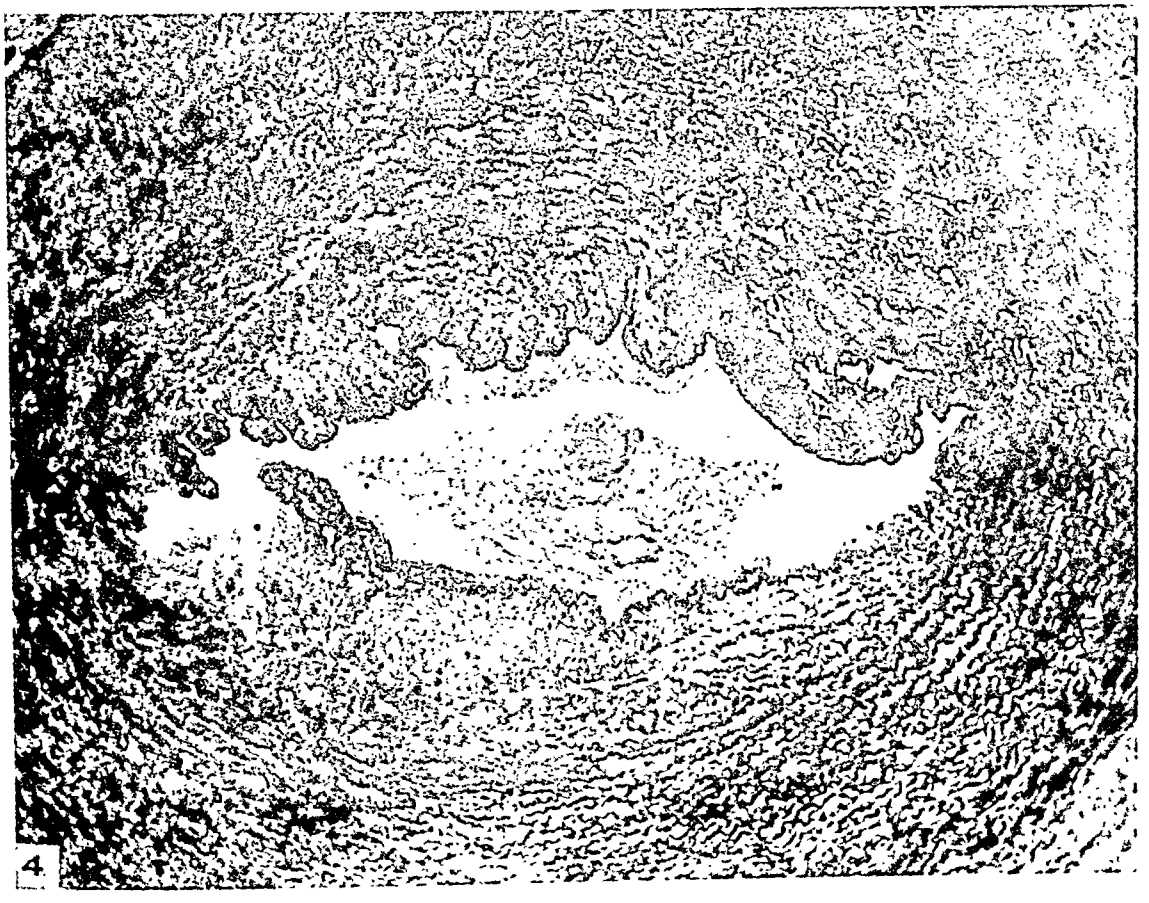
DESCRIPTION OF PLATES

PLATE 94

FIG. 1. Fetus 28 cm. in length. Low intimal mounds with fragmentation of the internal elastic lamina. Media loose, and poor in elastic tissue. Elastic tissue stain. × 105.

FIG. 2. Full-term fetus. Fragmentation of elastic lamina at base of intimal mound. Loose structure of media. Elastic tissue stain. × 185.





Jager and Wollenman

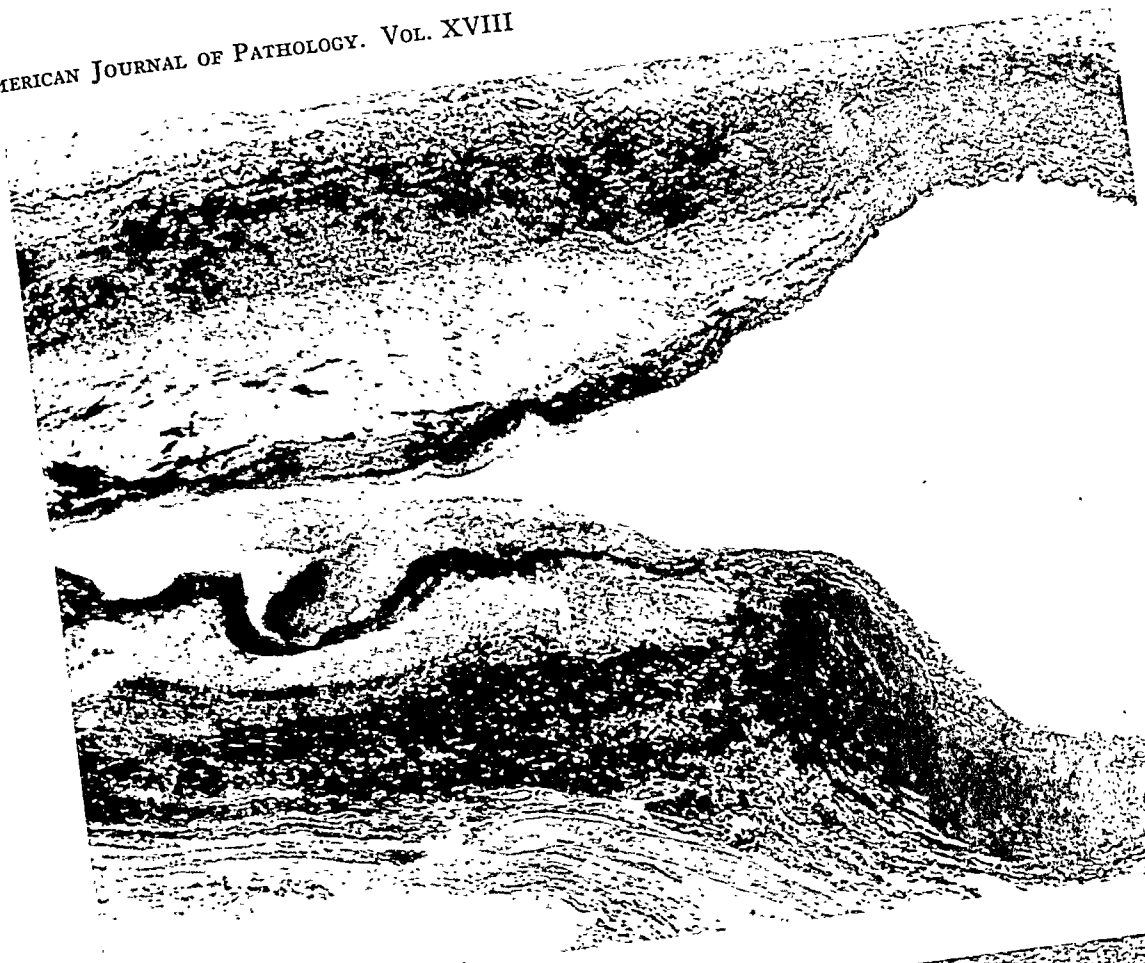
Closure of the Ductus Arteriosus

PLATE 95

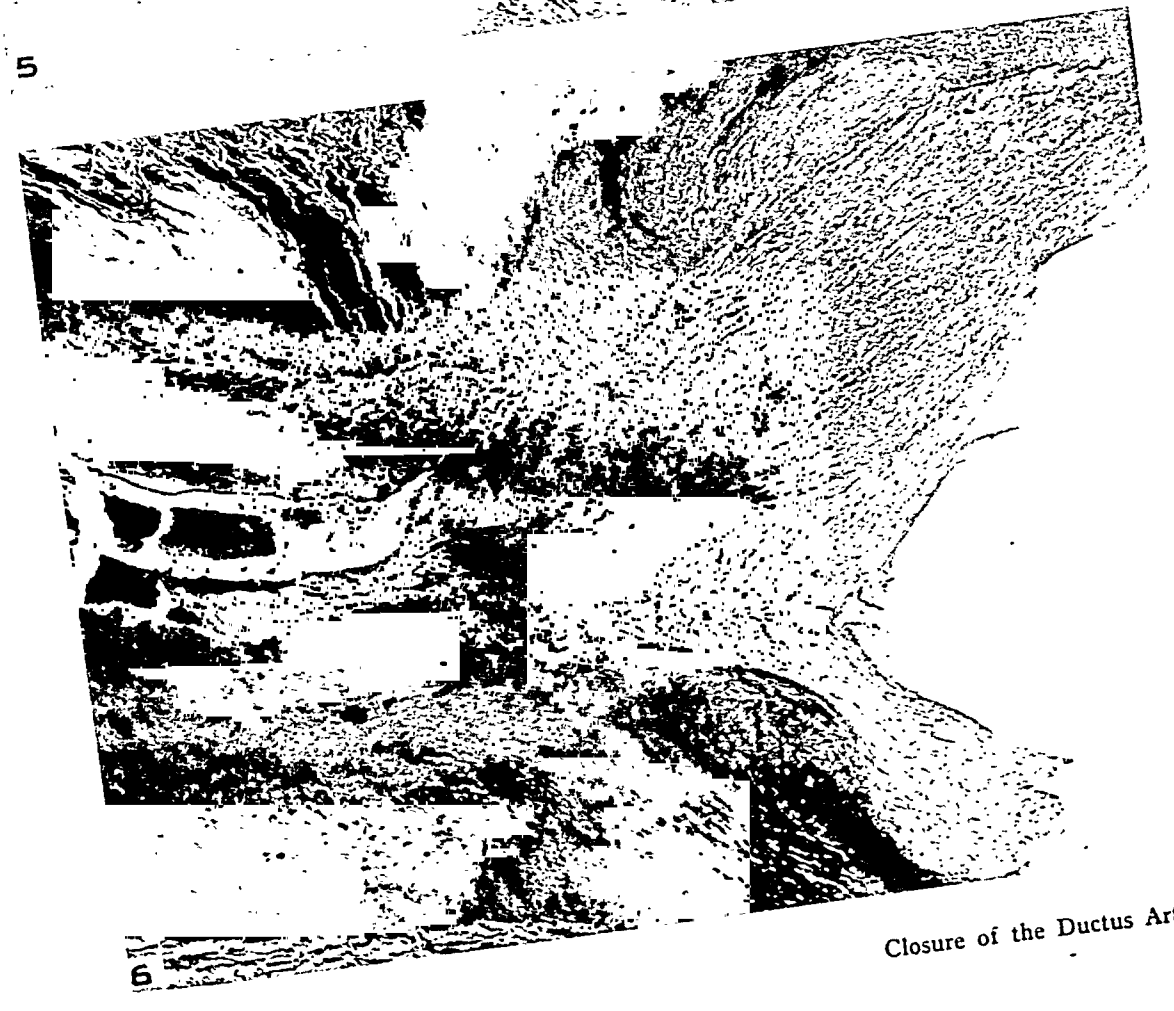
FIG. 3. Infant, 13 days of age. Intimal mounds of increased size crowned by connective tissue cap. The media is less loose in structure. Elastic tissue stain. × 35.

FIG. 4. Infant, 21 days of age. Lumen obstructed by connective tissue, suggesting an organized, recanalized thrombus. Increase in amount of elastic tissue of media. Elastic tissue stain. × 80.

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6

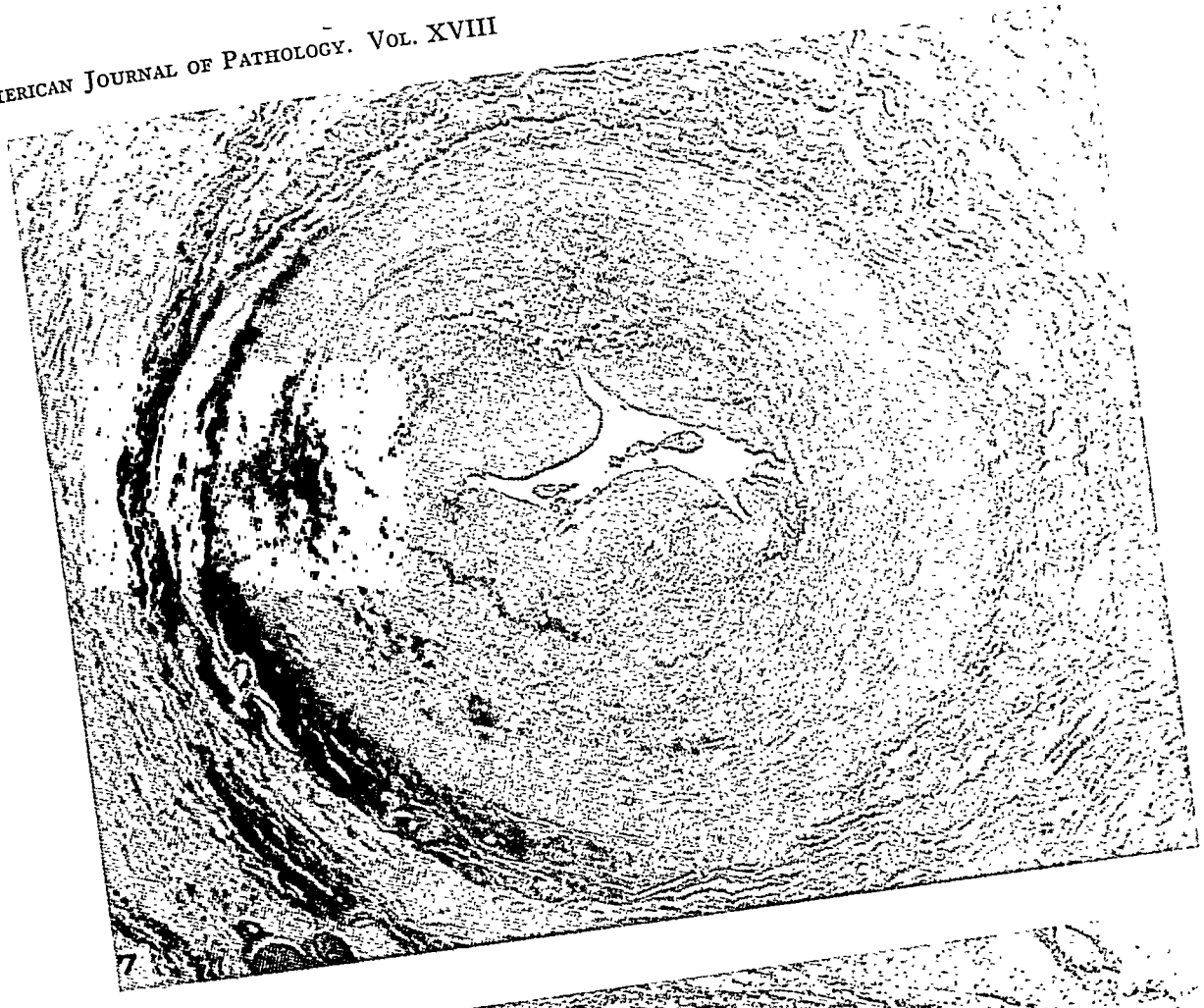
Closure of the Ductus Arteriosus

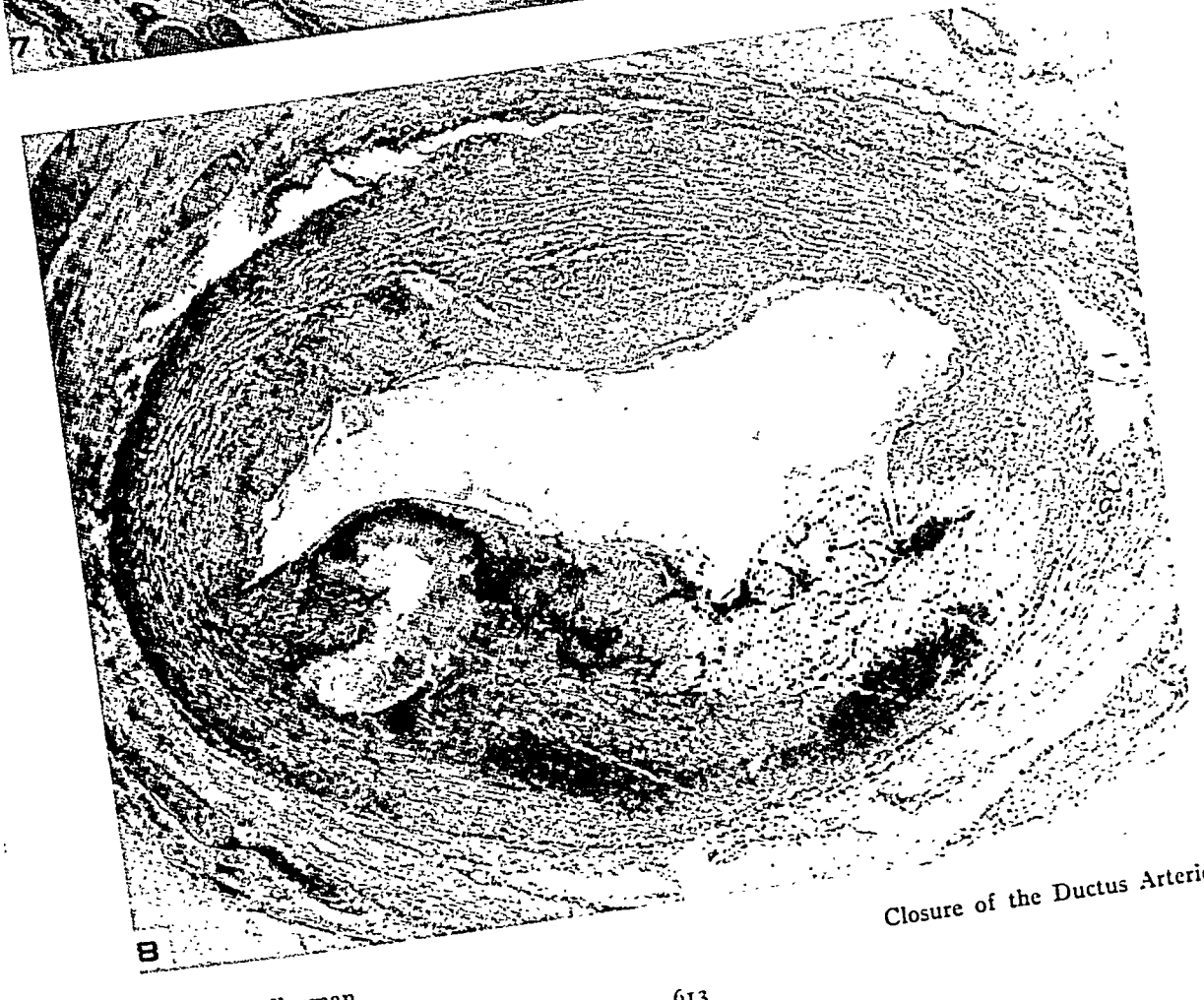
PLATE 96

FIG. 5. Infant, 22 days of age. Longitudinal section of obliterating aortic orifice of the ductus arteriosus showing aortic wall rich in elastic tissue while the ductus arteriosus is comparatively poor in elastic tissue and of looser structure. The streaming-in of elastic tissue, chiefly to the outer portion of the media, may be seen, but a slight amount is divided toward the intima to form the internal elastic lamina. Elastic tissue stain. × 25.

FIG. 6. Infant, 2 months of age: Longitudinal section of the obliterated aortic orifice of the ductus arteriosus with intimal connective tissue thickening at the site. × 35.

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Closure of the Ductus Arteriosus

PLATE 97

FIG. 7. Infant, 6 months of age. Marked degenerative changes. The internal elastic lamina remains distinct. The intimal mounds are now composed of collagen, connective and elastic tissue. Elastic tissue stain. × 35.

FIG. 8. Newborn infant. Dissecting aneurysm of the ductus arteriosus. Intimal mounds present, but not prominent. Hematoxylin and eosin stain. × 30.

TWO ANTAGONISTIC EFFECTS OF UNDERFEEDING ON THE ADRENAL CORTEX OF THE GUINEA PIG *

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As early as 1892, Martinotti [1] noted that when guinea pigs were starved for 3 or 4 days, the number of mitoses in the adrenal cortex increased from the usual 3 or 4 mitoses per section to as many as 20 to 25 per section; while the large majority of the mitotic figures under these conditions were normal, some seemed to be abnormal in character. Subsequently, however, Bonnamour [2] found no increase in mitotic activity in the suprarenal cortical cells of rats, guinea pigs, rabbits or cats when these animals were starved to such an extent that they died. Rondoni and Montagnini [3] also were unable to find any increase in mitotic activity in the adrenals of starved guinea pigs, and Jackson [4] similarly reported negative results in studying the adrenals of underfed rats. These were, to our knowledge, the only investigations in which the degree of proliferation of adrenal cortical cells was actually determined, and, as stated, all the more recent publications indicated that there was no increase in mitoses in the adrenal cortex of undernourished guinea pigs. However, an observation in humans apparently substantiates Martinotti's findings. Byrne [5] noted that the adrenals of soldiers who had died from underfeeding in German prison camps during the first World War were enlarged to 1.5 times the normal size; the enlargement, from the naked-eye appearance, appeared to be mostly cortical. Microscopic evidence of proliferation of the cells of the adrenal cortex was not recorded and it is not admissible to use the increased thickness of the adrenal cortex as a criterion of active growth processes, because such a change might depend upon the infiltration of the cortical cells with fatty substances or with carbohydrates.

A number of years ago it was noted by us that when guinea pigs were markedly underfed, the cells of the adrenal cortex became enlarged and there was an extraordinary increase in the number of mitotic figures. Approximate counts showed 100 or more mitoses in a section. In some of the mitoses the chromosomes became fragmented after reaching the metaphase. However, changes were not noted in all

*These investigations were carried out with the aid of grants from The International Cancer Research Foundation, from the Committee on Research in Endocrinology of the National Research Council and from the Jane Coffin Childs Memorial Fund for Medical Research.

Received for publication, November 10, 1941.



the controls (3.8 mitoses per section); the average weight loss at this time was 42.5 gm.

From these experiments it may be concluded that the mitotic count was increased as early as 2 days after the beginning of the underfeeding and continued to be elevated to approximately the same level for about 12 days of inanition. After 15 days of underfeeding the

TABLE I

The Effect of Severe Underfeeding on Mitotic Activity in the Adrenal Cortex of Male Guinea Pigs

Guinea pig no.	Period of underfeeding (days)	Total weight loss (gm.)	No. of mitoses per section*
1	2	25.0	13.1
2	2	20.0	6.2
3	4	30.0	5.2
4	4	55.0	18.1
5	4	25.0	8.6
6	4	25.0	15.0
7	4	40.0	5.1
		31.4 average	10.2 average
8	7	50.0	12.0
9	7	50.0	10.1
10	7	40.0	20.9
11	7	52.0	10.3
12	7	42.0	9.6
13	7	33.0	5.6
14	7	28.0	7.3
15	7	28.0	7.2
		40.4 average	10.4 average
16	10	30.0	4.4
17	10	50.0	17.5
18	12	35.0	4.4
19	12	40.0	10.9
		38.8 average	9.3 average
20	15	45.0	2.7
21	15	40.0	2.8
22	15	35.0	7.3
23	15	50.0	2.5
		42.5 average	3.8 average

* In 37 male guinea pigs used as controls, weighing 150 to 300 gm., the average number of mitoses per section was 3.2.

count had returned almost to normal. However, the cortex of guinea pigs markedly underfed for 15 days is not normal in appearance, and the decrease in mitotic count may be due to certain degenerative changes which have occurred at this time, rather than to a return to the normal state of the gland. The microscopic changes in cellular structure will be discussed in another part of this paper. Furthermore, the decrease in the number of mitoses after 15 days of underfeeding

experiments and for this reason publication of these experiments was deferred. It was in order to clear up, if possible, these variations in the results that the following investigations were undertaken.

Experiments

Seventy-two male and female guinea pigs, as a rule ranging in weight from 190 to 250 gm., but occasionally weighing as much as 250 to 300 gm., were used in additional experiments. These animals were fed a diet consisting of rabbit chow and greens, mixed in the usual proportions and containing the essential vitamins as well as carbohydrate, fat and protein, but they received diminished amounts of this feed for periods ranging from 2 to 20 days. The guinea pigs were weighed each morning and the quantity of food for any given day was regulated in accordance with the loss of weight of each animal during the previous 24 hours. Certain groups were underfed to a greater degree than others, as indicated in Tables I to IV. In one experiment, 10 male guinea pigs were underfed for 14 days and then refed for periods varying between 2 and 8 days, in order to determine whether the changes which occur in the adrenal cortex as a result of underfeeding are reversible. Mitotic counts were made in the manner described in a previous publication;[6] the average number of mitoses per section was determined by counting 25 sections through the central half of the adrenal gland.

In Table I are shown the results of mitotic counts of the adrenal cortex in 23 underfed male guinea pigs whose weight loss ranged between 20 and 55 grams. The controls for this experiment consisted of 37 normally fed male guinea pigs, selected in such a manner that their weights ranged from that of the heaviest animal at the onset of underfeeding (300 gm.) to that of the lightest guinea pig at the completion of the period of underfeeding (150 gm.). The average number of mitoses per section in these 37 controls was 3.2. It can be seen that as little as 2 days of marked underfeeding results in an elevation of the number of mitoses. In the 7 male guinea pigs underfed for 2 and 4 days, the average mitotic count was 10.2, with a range of variation between 5.1 and 18.1. The average weight loss in these guinea pigs was 31.4 gm. After 7 days of underfeeding the average mitotic count for 8 male guinea pigs was only slightly higher than after 2 to 4 days of inanition (10.4 mitoses per section), and the average weight loss was also somewhat higher, being, on the average, 40.4 gm. After 10 to 12 days of underfeeding the average mitotic count was 9.3 and the average weight loss was 38.8 gm., while after 15 days of underfeeding the average number of mitoses was only slightly above that of

The degree of underfeeding in this experiment was similar to the corresponding experiment with male guinea pigs (Table I). It is evident from a comparison of the figures in Tables I and III that underfeeding causes a very much greater increase in mitoses in females than

TABLE III

The Effect of Severe Underfeeding on Mitotic Activity in the Adrenal Cortex of Female Guinea Pigs

Guinea pig no.	Period of underfeeding (days)	Total weight loss (gm.)	No. of mitoses per section*
1	4	37.0	14.9
2	4	37.0	22.8
		37.0 average	18.9 average
3	7	35.0	13.4
4	7	30.0	19.2
5	7	30.0	91.3
6	7	35.0	15.9
7	7	35.0	12.3
8	7	35.0	24.9
9	7	27.0	13.1
10	7	35.0	11.6
11	7	40.0	7.4
12	7	35.0	10.9
13	7	28.0	9.2
		33.2 average	20.8 average
14	10	25.0	109.0
15	10	25.0	22.0
16	10	30.0	21.1
17	10	25.0	56.9
		26.3 average	52.2 average
18	12	30.0	44.6
19	12	45.0	116.0
20	12	35.0	197.2
		36.7 average	119.3 average
21	15	50.0	3.0
22	15	35.0	10.8
23	15	85.0	11.4
24	15	80.0	11.2
		62.5 average	9.1 average
25	20	80.0	0

* In 43 female guinea pigs used as controls, weighing 150 to 350 gm., the average number of mitoses per section was 4.9.

in males. In 2 guinea pigs, underfed for 4 days, the average weight loss was 37 gm. and the average mitotic count was 18.9 per section, as compared with 10.2 mitoses per section in males underfed for a similar period. After 7 days of underfeeding the mitotic count in females rose on the average to 20.8 per section; the average weight loss of these 11

may be due in part to the fact that the loss of weight per unit of time has been less in these guinea pigs than in those examined at earlier periods.

In Table II are shown the results of an experiment in which male guinea pigs were underfed for 4 to 10 days, in such a manner that the loss in weight did not exceed 20 gm. In this experiment 14 guinea pigs lost an average weight of 14.3 gm. Fourteen normally fed male animals served as controls; they ranged in weight between 170 and 200 gm. and had an average mitotic count of 3.6. The average mitotic count in the 7 guinea pigs underfed from 4 to 7 days was 0.9, while in the

TABLE II

The Effect of Moderate Underfeeding on Mitotic Activity in the Adrenal Cortex of Male Guinea Pigs

Guinea pig no.	Period of underfeeding (days)	Total weight loss (gm.)	No. of mitoses per section*	
1	4	10.0	0.2	
2	7	5.0	0.8	
3	7	20.0	3.8	Average no.
4	7	10.0	0.8	of mitoses per
5	7	5.0	0	section, 0.9
6	7	15.0	0	
7	7	15.0	0.4	
8	10	15.0	4.0	
9	10	20.0	0	
10	10	20.0	1.8	Average no.
11	10	15.0	0.2	of mitoses per
12	10	20.0	0.8	section, 1.6
13	10	15.0	4.4	
14	10	15.0	0.2	
		14.3 average	1.2 average	

* In 14 male guinea pigs used as controls, weighing 170 to 200 gm., the average number of mitoses per section was 3.6.

7 males underfed for 10 days it was 1.6. The average number of mitoses per section in all 14 moderately underfed males was 1.2. It appears, therefore, that there is some depression of karyokinetic activity in guinea pigs underfed to a moderate degree; this depression seems to be somewhat greater during the first 7 days of underfeeding than after the 10-day period, as is evidenced by the fact that there were 6 animals in which there was less than one mitosis per section in the former group and only 4 such guinea pigs in the latter group. No marked histologic changes in the adrenal cortex were noted in this series.

Table III shows the results of counts carried out in markedly underfed female guinea pigs. The controls for this experiment were chosen in the same way as those for the previous two experiments and consisted of 43 female guinea pigs with an average count of 4.9 mitoses.

such a way that they lose approximately 60 gm. (about 25 per cent of their original weight), the adrenal cortex shows a low mitotic count and manifests signs of exhaustion. When these animals are subsequently refed, the cortical cells go through a process of recovery characterized at first by increased mitotic activity. This increase in mitotic proliferation was at its height when the animals had regained approximately 50 per cent of their original weight loss. It was first noticed when 25 per cent of the lost weight was recovered, and it disappeared after about 80 per cent of the original loss in weight had been regained.

TABLE IV

The Effect of Underfeeding and Subsequent Refeeding on Mitotic Proliferation in the Adrenal Cortex of Male Guinea Pigs

Guinea pig no.	No. of days refed	Weight lost after 14 days of underfeeding (gm.)	Weight regained after refeeding (gm.)	No. of mitoses per section
1	0	60		4.3
2	0	65		2.5
				3.4 average
3	2	60	15 (25%)	13.7
4	2	50	5 (10%)	4.4
				9.1 average
5	4	60	30 (50%)	16.7
6	4	65	35 (53.8%)	25.4
				21.1 average
7	6	70	60 (85.7%)	5.6
8	6	50	40 (80%)	14.3
				10.0 average
9	8	50	60 (120%)	2.3
10	8	40	50 (125%)	4.2
				3.3 average

HISTOLOGIC CHANGES IN THE ADRENAL CORTEX IN UNDERFED GUINEA PIGS

The histologic changes which were noted in guinea pigs subjected to underfeeding are shown in Figures 1 to 4. In the adrenals of normal guinea pigs, a representative specimen of which is shown in Figure 1, the cortical cells in the outer third of the zona fasciculata contained a lipoid or fatty substance, which appeared as large vacuoles when the tissue was prepared by the paraffin technic. These vacuoles occupied a large portion of the cell and caused a compression and reduction in the size of the nucleus. When guinea pigs weighing 200 to 250 gm.

guinea pigs was 33.2 gm. The mitotic count in males underfed for a similar 7-day period was 10.4. In 4 female guinea pigs underfed for 10 days the average mitotic count rose to 52.3 and the average weight loss was 26.3 gm.; 3 females underfed for 12 days had, on the average, 119.3 mitoses per section and an average loss in weight of 36.7 gm. Males similarly underfed for 10 to 12 days showed an average of 9.3 mitoses per section. When the 7 female guinea pigs underfed for 10 and 12 days are combined into a single group, the average mitotic count is 81.0. Further, 4 females were underfed for 15 days and showed an average weight loss of 62.5 gm.; the average mitotic count for this group was 9.1 per section. In males markedly underfed for the same length of time the mitotic count was 3.8 per section. Finally, after 20 days of underfeeding, 1 guinea pig had lost 80 gm. in weight and had a mitotic count of 0. Here also, as in the experiment in which marked inanition was produced in males, the return of mitotic proliferation to a low state of activity does not indicate a return to the normal state of the adrenal cortex, but rather to a state of exhaustion of the adrenal cortical cells. It can be seen from this experiment that while underfeeding causes a much greater rise in mitoses in females than in males, the character of the curves representing the changes in mitotic activity as a function of time are somewhat similar in the two sexes.

In a fourth experiment (Table IV) we attempted to ascertain whether the changes in the adrenal cortex resulting from marked underfeeding were reversible. Ten male guinea pigs were underfed for 14 days. Two of these animals were autopsied as controls at the end of this period of inanition; they had lost, on the average, 62.5 gm. in weight and showed a mitotic count of 3.4 per section. Two of the guinea pigs were refed for 2 days; one of these regained 15 gm. of its lost weight and had a mitotic count of 13.7, while the other regained only 5 gm. and had a mitotic count of 4.4. The average count for these two animals was 9.1. Two other males were refed for 4 days and regained 30 and 35 gm. respectively; their mitotic count was, on the average, 21.1. Two guinea pigs refed for 6 days regained 60 and 40 gm. of their original loss in weight and showed a mitotic count of 5.6 and 14.3 respectively; the average mitotic count for these two animals was 10.0 per section. Finally, two males were refed for 8 days, at which time they had regained completely their original loss in weight, as well as an additional 10 gm. The average number of mitoses in these two guinea pigs was 3.3.

From this experiment, it may be concluded that when male guinea pigs weighing about 250 gm. are underfed for a period of 14 days, in

the cells of the zona glomerulosa and some of the outermost cells of the zona fasciculata showed little evidence of damage, and it is probably from these cells that the cortex regenerates when the process of refeeding is instituted. This state of exhaustion occurs earlier in males than in females; in certain males it was observed as early as after 12 days of underfeeding, whereas in females it was often just beginning to appear after 15 days of underfeeding, when the mitotic count in some of these animals was still somewhat elevated.

Finally, it was noted that in many of the cells undergoing mitotic division this process did not progress beyond the metaphase. In these cells the chromosomes frequently became fragmented and particles of chromatin of various sizes and shapes were spread haphazardly throughout the cytoplasm. When mitotic activity was at a maximum, as many as 20 to 30 per cent of the cells undergoing karyokinesis showed this dispersed type of chromatin. However, even if these cells were omitted from the mitotic counts, the latter would still be considerably elevated.

Discussion

These experiments may account for the differences in the results obtained in some previous investigations. Thus, the very high degree of proliferation, the frequent occurrence of abnormal mitoses in some cases and the lack of these changes in others may have been due to differences between male and female guinea pigs or to differences in the length of time during which the animals were exposed to underfeeding. These factors may explain the lower counts found by Martinotti.[1] Moreover, Martinotti did not state to what degree his animals were underfed; on the other hand, Bonnamour,[2] as well as Rondoni and Montagnini,[3] apparently starved guinea pigs to such an extent that the adrenal glands were in a state of exhaustion at the time of examination.

The explanation of the failure of Jackson[4] to find an increase in proliferative activity in the rat as a result of inanition may be due to factors other than the degree of underfeeding, since this investigator produced undernourished states of varying degrees and for varying periods of time. However, the responsiveness of various endocrine glands is different in the rat than in the guinea pig. Thus Loeb[7] and Loeb and Bassett,[8] as well as Thurston,[9] have shown that the thyroid gland of the rat responds much less actively to the stimulating effects of anterior pituitary extract than does this gland in the guinea pig, and in some unpublished experiments Blumenthal has observed that the adrenal cortex of the rat likewise responds to a lesser degree than

were underfed sufficiently to bring about a 15 to 20 per cent loss in weight in a period of 2 to 4 days, these vacuoles began to disappear and the cortical cells enlarged. The nuclei also increased in volume and became well rounded and vesicular. At this time mitotic figures appeared in increased numbers. Some mitotic divisions occurring in a single field are shown in Figure 2, which represents this stage of the process of inanition; in this figure only one vacuole can be seen. In Figure 3 is shown the condition which was present when the state of undernourishment was maintained for a period of 10 days. The vacuoles have entirely disappeared, the cortical cells and their nuclei continue to be enlarged and mitoses are numerous.

During the process of marked inanition this fatty or lipoid material disappeared as mitotic activity increased. In males, a few vacuoles usually persisted, but in females they were usually absent when mitotic activity was at a maximum. This finding corresponds to the difference in the degree of mitotic activity observed in the two sexes. In guinea pigs which were only moderately underfed, so that the loss in weight in the first 10 days ranged between 5 and 20 gm., the number of mitoses, instead of increasing, showed a definite decrease. In accordance with expectations, the vacuoles did not disappear from the cortical cells. In this case, therefore, the pure effect of underfeeding predominated, with a resulting decrease in proliferative activity. In male guinea pigs which were underfed and subsequently refed there was, at first, a complete absence of vacuoles after 14 days of underfeeding and the cells showed signs of exhaustion, as described below. With refeeding, there was a lag period of about 4 days before vacuolar inclusions were noted, and in the period from 4 to 6 days, although present, they were in smaller numbers and of diminished size as compared with normally fed guinea pigs. After 8 days of refeeding, the normal number of vacuoles reappeared and their size also increased; accordingly, there was no longer any increase in the number of mitoses. From these observations it may be concluded that a direct relation exists between the increase in size of cells and augmented mitotic activity on the one hand, and the disappearance of fatty substances on the other hand.

When marked inanition was maintained for about 15 days, signs of exhaustion set in. The vacuoles were absent but the cells of the zona reticularis and deeper zona fasciculata underwent degeneration, the nuclei becoming either pyknotic or karyorrhetic and many of the cells disintegrated completely. The more peripheral cells of the zona fasciculata were swollen and contained a frothy albuminous material, while the nuclei were compressed and pyknotic. At this stage only

in mitoses. But, if fat droplets are deposited in these cells in the process of milk production, mitoses cease, as a rule; they are found usually only in cells in which these droplets are as yet lacking. It is known that in the cortex of the adrenal gland fatty and lipoid substances, and to a less extent also glycogen, are found in quantities which vary under different conditions. Considering all these facts, it is reasonable to assume that it may be the deposit of certain fatty or lipoid substances in the cortical cells which diminishes the mitotic proliferation, and that removal of these substances might lead to an increase in mitoses. The observations which we have recorded here support this interpretation of the effect of undernourishment in cell proliferation in the adrenal cortex. Whenever a marked increase in the number of mitoses occurs in the adrenal cortex, fat or lipoid vacuoles have disappeared in the cortical cells in which proliferation may take place. Moreover, the differences in the degree of mitotic activity which are found in underfed male and female guinea pigs are in agreement with this interpretation. The deposit of this substance in the cells brings about changes unfavorable to mitotic proliferation, which otherwise would occur under the influence of stimulating hormones. But in addition to this specific factor, the general effect of undernourishment, consisting in a diminution or cessation of mitotic activity, is also found. If the undernourishment exceeds a certain degree or extends over a longer time, the number of mitoses in the cortical cells diminishes again, or ceases altogether. Then if a refeeding occurs, there may be observed a stage when the injurious effect of undernourishment is removed and the cell is ready to respond again to hormonal stimulation with an increased mitotic proliferation, a period which will be terminated by the reappearance of fatty deposits or perhaps also under the influence of some other changes. As to the abnormal character of some of the mitoses which appear in undernourished guinea pigs, it may be suggested that this is due to the combined effect of the removal of the fatty cell-inclusions, which is favorable to the development of mitoses, and of the injurious action which starvation otherwise exerts on the cell metabolism; presumably the deficiencies due to starvation of the cells cause degenerative conditions during the latter stages of the mitoses.

In experiments which one of us is at present carrying out, it has been noted that when acid extract of bovine anterior hypophysis is administered to guinea pigs, there is a marked loss of fat vacuoles with a coincident increase in the size of the cortical cells and a marked rise in mitotic activity. Also, when mitotic proliferation is very pronounced, abnormal mitoses, with fragmentation of chromatin, similar to that

does the adrenal of the guinea pig, when animals of these two species receive equal amounts of acid extract of bovine anterior hypophysis per unit of weight. Further differences in the mode of reaction of the adrenal glands of these two species are indicated by the fact that bacterial toxins and particularly diphtherial toxin, which produce marked pathologic changes in the adrenal cortex of the guinea pig, have no grave effect on this gland in the rat.

As to the mechanism underlying the remarkable increase in proliferation in the adrenal cortex caused by undernourishment under certain conditions, the following facts have to be considered:

(1) Loss of weight of organs, if it reaches a certain degree, has the tendency to lower the proliferative activity of organs and tissues; moreover, it tends to inhibit the increase caused by some stimulating factors. This is exemplified by the behavior of the thyroid gland, where undernourishment decreases the number of mitoses occurring in normal organs and where it diminishes or prevents also the increase in mitoses usually caused by administration of potassium iodide, as shown by Gray and Loeb [10] and Rabinovitch,[11] as well as by Rabinovitch and Gray.[12] However, the intensity of the effect of undernourishment on the proliferative activity varies in different organs and tissues; it is less in the epidermis than in the thyroid of the guinea pig (Loeb, Haven, Genther and Friedman [13]). In no organ other than the adrenal has it so far been observed that undernourishment causes an increase in the number of mitoses.

(2) The proliferative activity of tissues is strongly influenced by certain secondary differentiations, which take place either in the course of embryonal development or by changes which occur in the connective tissue during extra-uterine life. The latter are associated with the production of paraplastic structures and the deposit of various substances within the cells composing these tissues. Thus the production of polymorphonuclear leukocytes is connected with a cessation of mitoses. In the epidermis, processes which precede keratinization likewise cause a cessation of mitotic activity; stimulation of such cells may lead to a production of amitoses instead of mitoses. In the guinea pig, the transformation which the granulosa cells of the ovarian follicles undergo during the process of maturation, consisting in an increase in cytoplasm which stains strongly with eosin, is associated with a cessation of proliferative activity, which can, to a certain extent, be resumed when the mature granulosa cells become converted into corpus luteum cells (Loeb [14]). Very instructive in this respect is the behavior of the mammary gland of the mouse. Here, under the influence of certain hormones, proliferation takes place, as is indicated by an increase

vacuoles, an increase in the size of the cortical cells and the appearance of abnormal mitotic figures in which there is a dispersion of chromatin after the metaphase has been reached. The disappearance of fat vacuoles in female guinea pigs is practically complete when mitotic activity is at a maximum, while in males there may be some remaining vacuoles at this time. Correspondingly, the degree of mitotic response to severe underfeeding is considerably greater in females than in males.

3. It has also been shown that these changes are completely reversible, since when male guinea pigs are underfed for a sufficiently long period to bring about a state of exhaustion in the adrenal cortex, subsequent refeeding results first in a gradual increase in the number of mitotic figures to above the normal, and this condition is followed by a return of the cortex to the normal histologic state with a normal mitotic count.

4. When guinea pigs are only slightly underfed for periods lasting from 4 to 10 days, there is a suppression of mitotic activity rather than the increased proliferative activity observed with more marked degrees of inanition.

5. Under all conditions observed so far there was an inverse relation between the frequency of fatty or lipoid inclusions in the cortical cells and the frequency of mitotic proliferation of these cells.

6. The various effects of underfeeding on the adrenal cortex of guinea pigs can be explained if we assume that the removal of lipoid or fatty cell-inclusions eliminates a factor which inhibits mitotic proliferation (which otherwise would take place under the influence of certain hormones) and that this removal is responsible for the marked increase in mitoses which is observed in these cells under certain conditions of underfeeding. The increase in mitosis in the adrenal cortex of underfed guinea pigs must therefore be regarded as an indirect effect. It must also be assumed that underfeeding as such has the opposite effect, inasmuch as it diminishes the proliferative activity. Under different conditions one or the other of these effects predominates.

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which has been described in these investigations, are observed. When this extract is administered to older guinea pigs, whose adrenal cortical cells contain a greater number of these fatty inclusions than those of younger animals and in whose cells other paraplastic substances such as pigments may be present, the degree of mitotic response to hypophyseal extract is markedly diminished. The difference in the degree of proliferative response to underfeeding in male and female guinea pigs may perhaps be due to differences in their ability to metabolize these lipoid or fatty substances.

It thus appears probable that the presence of certain fatty or lipoid substances in cortical cells inhibits their proliferation, which otherwise would take place under the influence of hormones, and that, conversely, the discharge of lipoid or fatty materials from the adrenal cortex may lead to an increase in mitotic activity on the part of the cells of this gland. That this interpretation is correct seems more probable than the assumption that the changes in the fat or lipoid content are merely a concomitant of the increase in mitotic activity, but not its direct cause. Such an assumption is unlikely in view of the general parallelism between the elimination of these paraplastic substances and the increase in mitotic proliferation, and in view also of other factors which point to an inactivating effect of cell differentiation and of intracellular accumulation of paraplastic substances as already discussed.

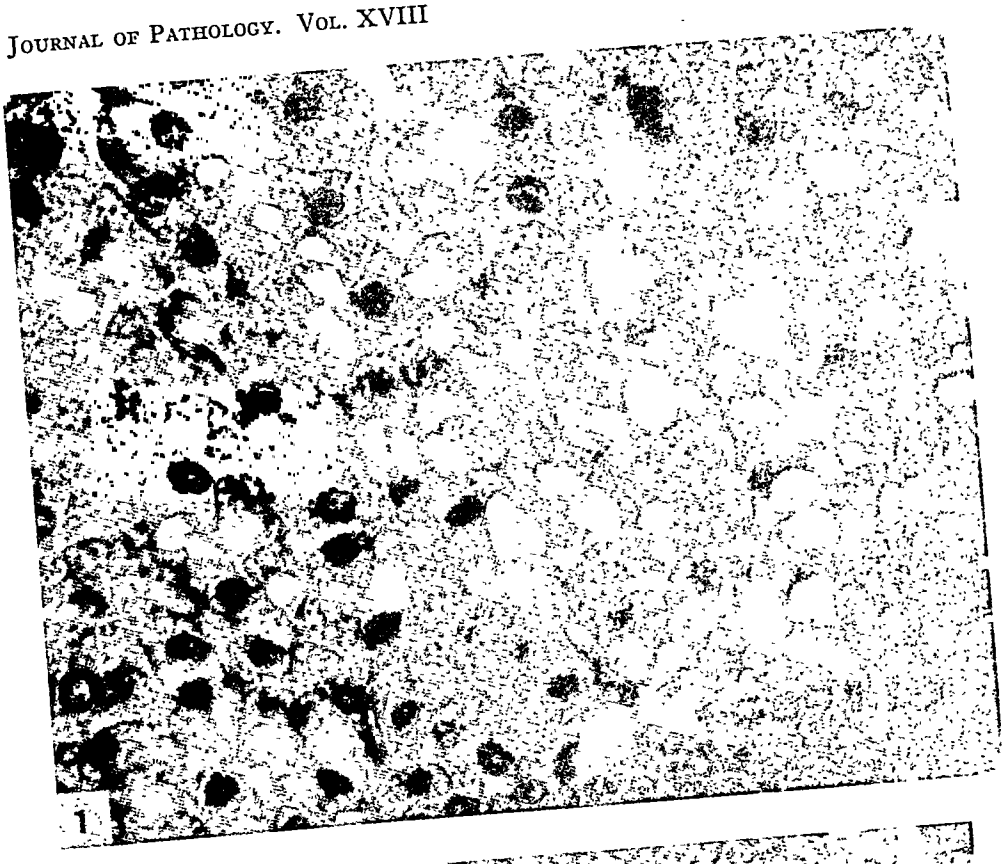
It may then be concluded that the observations reported by us in this paper agree with the interpretation which we have given. The theory here presented may explain also some observations of Selye,[15] who noted that during the first 24 hours of the so-called alarm reaction a marked hyperplasia of the adrenal cortex occurs, although it is not stated that this is due to an actual increase in the mitotic activity. The increase in the size or number of cortical cells is accompanied by a loss of lipoid inclusions in the cortical cells, and in this case, too, the removal of these cell inclusions may be the cause of the increase in the number of cells.

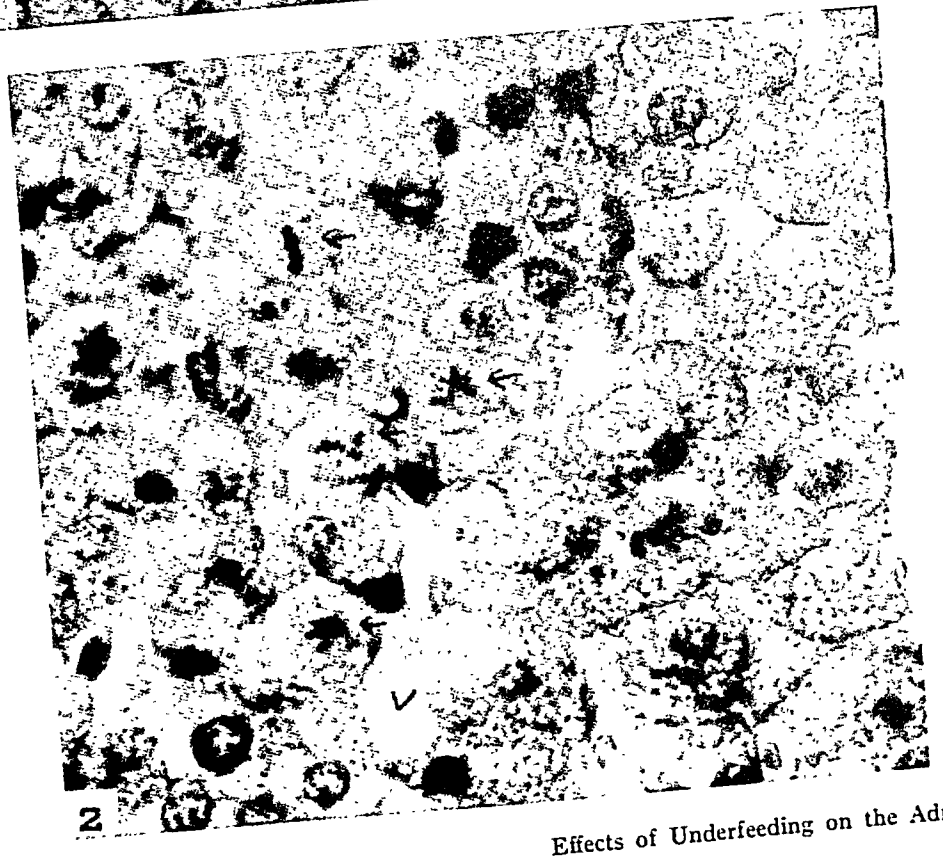
Summary

1. Marked inanition produces an increase in mitotic activity of the adrenal cortical cells, which in males is most pronounced between the fourth and seventh days of underfeeding; the increase in mitoses is much greater in females than in males; the maximum in the former is reached after 10 to 12 days of undernourishment, when the number of mitoses per section may exceed 100.

2. The microscopic changes in the adrenal cortex, due to underfeeding, are characterized by a marked decrease in fatty or lipoid

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Effects of Underfeeding on the Adrenal Cortex

Blumenthal and Loeb

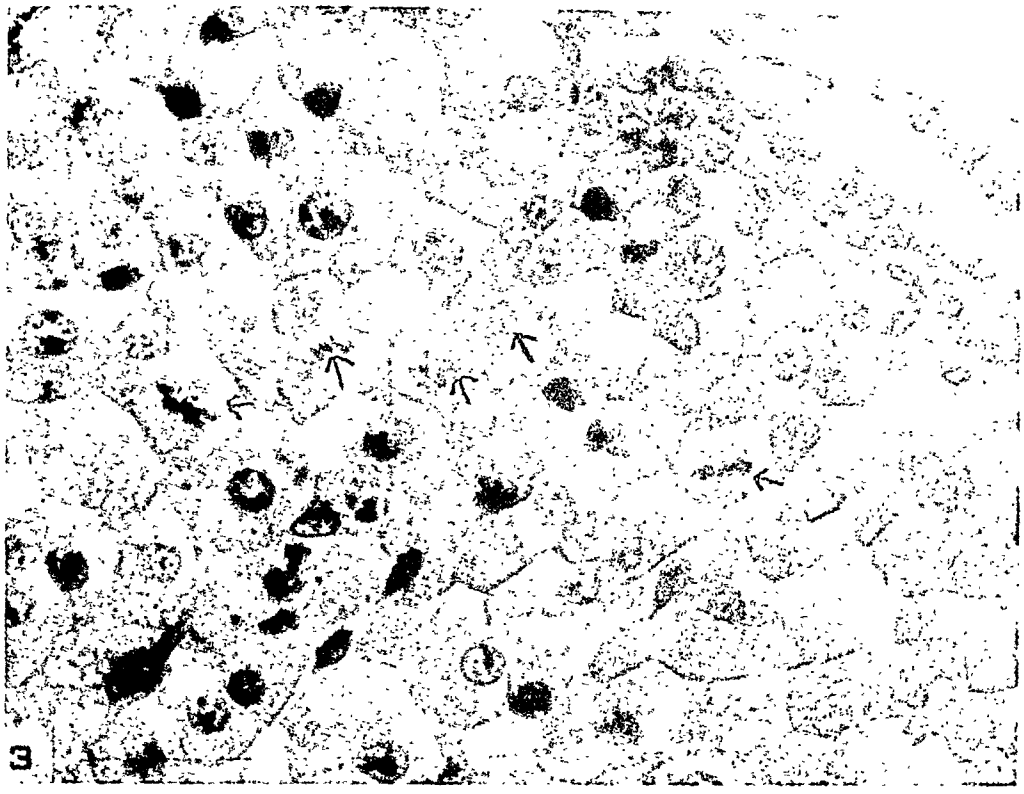
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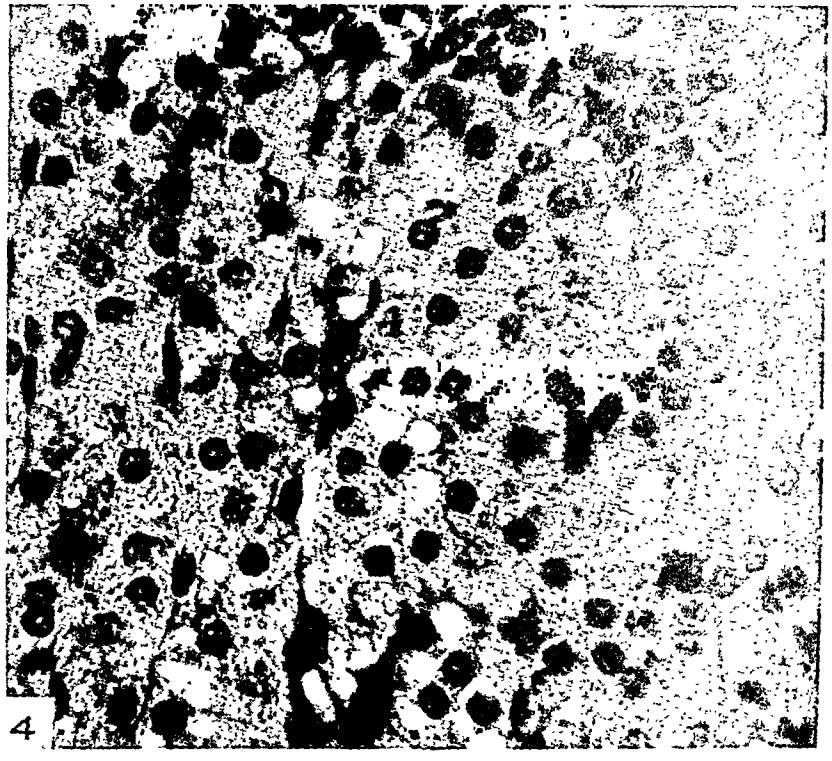
DESCRIPTION OF PLATES

PLATE 98

FIG. 1. Section of adrenal cortex of normal female guinea pig weighing 250 gm. Lipoid vacuoles are present in many of the cells of the outer zona fasciculata. There are no mitoses. × 440.

FIG. 2. Section of adrenal cortex of female guinea pig underfed for 4 days; weight loss was 40 gm. (from 245 to 205 gm.). The lipoid vacuoles have disappeared from all but one cell (v) in this region of the outer zona fasciculata. A number of mitotic figures are present; these are indicated by arrows. × 440.





Blumenthal and Loeb Effects of Underfeeding on the Adrenal Cortex

PLATE 99

FIG. 3. Section of adrenal cortex of female guinea pig underfed for 10 days; weight loss was 60 gm. (from 250 to 190 gm.). The lipoid vacuoles have completely disappeared from this region of the outer zona fasciculata. A number of mitotic figures are present; these are indicated by arrows. × 440.

FIG. 4. Section of adrenal cortex of a male guinea pig slightly underfed for 7 days; weight loss was 15 gm. (from 240 to 225 gm.). The lipoid vacuoles are still present in the outer zona fasciculata; no mitotic figures are visible and the cells and nuclei are somewhat smaller. × 440.

FUNCTIONING ISLET CELL CARCINOMA WITH METASTASES TO LIVER *

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Spontaneous hypoglycemia has been established as usually secondary to islet cell tumors, either benign or malignant. Among these, the cases in which metastases have occurred have been surprisingly few. Frantz,[1] in a recent review of 96 tumors of islet cells, could find only 5 cases with proved metastases.[2-6] These are summarized in Table I. In addition, she listed 21 cases suspected of being malignant on the basis of capsule or blood-vessel invasion, but no report of metastases occurring in these patients has appeared in the literature to date (August 22, 1941).

Four additional cases,† including one case studied and autopsied in this hospital, of proved, functioning islet cell carcinoma with metastases have come to our attention. One was the recent case of Flinn, Beatty, Ginsberg and Hemsath,[8] giving a typical history of hypoglycemic attacks over a period of 5 months. Blood-sugar determinations as low as 20 mg. per cent were obtained. There was no operation. At autopsy an islet cell carcinoma was found in the head of the pancreas with metastases to the liver and lymph nodes. No assay of insulin was made. The pituitary was grossly normal. There were no other essential findings.

Jacobsen,[9] in a brief abstract, recorded in the history of a male, 36 years of age, that on the finding of glycosuria, a diagnosis of diabetes was made. On insulin regime he developed signs of shock with blood-sugar determinations as low as 25 mg. per cent. In the following year, the taking of sugar warded off attacks. At operation, an inoperable tumor, the size of a small grapefruit, replaced the head of the pancreas. Many nodules were seen in the liver. The diagnosis was first made from one of these. Biopsy showed a striking resemblance to islet cells, and beta granules were demonstrated. These findings were confirmed by autopsy. No assay of insulin was made. No photomicrographs were included as the case was to be reported in detail at a later date. We

* Received for publication, August 30, 1941.

† Not included among these is the case reported by Ballinger [7] of a tumor involving liver, right lung, right hilar lymph nodes, adrenal glands, spleen, kidneys, heart and a subcutaneous nodule. The pancreas showed no tumor. He interpreted the findings as an islet cell carcinoma arising in aberrant pancreatic tissue in the liver. There are so many peculiarities in the general picture that it is difficult to accept this case as one of proven islet cell carcinoma with metastases.

a tumor in the tail of the pancreas with multiple nodules in the liver. The pancreatic tumor was excised and tissue for biopsy removed from one of the hepatic nodules. The postoperative course was complicated at first by bronchopneumonia; persistent purulent discharge from the pancreatic wound; thrombophlebitis and abscess of the right arm, probably associated with continuous intravenous therapy; marked anorexia; persistent and extraordinary shortness of breath, and rapid respiration as well as persistent high pulse rate. Physical examination and roentgenograms of the chest yielded no findings which entirely explained the cardio-respiratory findings. Finally, about 6 weeks after operation, she seemed to be improved and was allowed to sit in a chair for short periods. On November 3, 1939, she suddenly developed extreme dyspnea, rapidly became unconscious, pulseless and deeply cyanotic, without râles being heard in the chest, and died in 15 minutes.

The behavior of the blood sugar in the postoperative period merits attention. During the first week, on constant intravenous glucose, the values were at a high normal level (90 to 120 mg. per cent). When intravenous glucose was stopped, the values gradually increased to 250 mg. per cent, at which time glycosuria to the extent of 35 gm. per 24 hours was obtained. Protamine zinc insulin was administered for 1 month in dosage of 12 to 16 units before breakfast. With such treatment the glycosuria cleared up and the blood-sugar values were maintained at a satisfactory level. During the last 10 days of life no insulin was given, and on a carbohydrate intake of 150 to 200 gm. daily, blood sugar ranged from 110 to 150 mg. per cent.

Pathological Report Upon Surgical Specimen

(The following report was made by Dr. Shields Warren.) The surgical material consisted of a nodule from the pancreas and a specimen from the liver. Thin blocks were fixed in the following fluids: Zenker's, Helly's, and Bouin's as well as in a 4 per cent solution of formaldehyde and in absolute alcohol. Routine stains were made with eosin and methylene blue and phosphotungstic acid hematoxylin. Special stains were also used as indicated.

The pancreatic mass measured 1.8 by 1.8 by 1.3 cm. and was slightly irregular in outline with a rim of adherent pancreatic tissue. Microscopically the pancreatic tumor was composed of polyhedral cells arranged in various ways. Some grew in sheets which had the architectural relationship of islet cells. Elsewhere many elongated, irregular cells with almost clear cytoplasm tended to be arranged in rows with the long axes of the cells at a right angle to the row. They resembled one side of a long duct. These two types of cells merged into one another. More rarely, larger, cuboidal or columnar cells with almost clear cytoplasm tended to form acini and occasionally to secrete mucinous material. No cells resembling those in normal acini were seen. The nuclei of the three types were essentially similar, either round or oval, with finely divided chromatin. Mitoses were rare. The tumor cells penetrated through the capsule and invaded the surrounding pancreatic tissue. Blood-vessel invasion was not seen. Groups of tumor cells were widely separated by bands of dense, structureless hyaline ma-

have reviewed the slides from this case and have confirmed the diagnosis (through the permission and kindness of Dr. V. C. Jacobsen).

Through the kindness and permission of Dr. Paul R. Cannon we have been able to review the slides and confirm the diagnosis of an additional case, the histological details of which are thus far unpublished although it has been mentioned in other reports.[10,11] A male, 36 years of age, had spontaneous hypoglycemic attacks for a few months. Fasting blood sugars as low as 16 and 23 mg. per cent were obtained. No glycosuria or ketonuria was observed. Intravenous glucose immediately stopped the attacks. The glucose requirement was 600 gm. daily. The body weight increased from 57.1 to 64.3 Kg. in 3 months. Exploratory laparotomy revealed many tumor nodules in the liver and a mass in the pancreas. Biopsy of a liver nodule was performed. At autopsy a carcinoma of the pancreatic islet cells was found, with metastases to the liver, peripancreatic, periaortic and mediastinal lymph nodes, one adrenal, spine, lungs and pleura. Assay of insulin was inconclusive.

Report of Case

F. H., a female patient, 48 years old, was studied in this hospital. Only a brief review of the clinical findings is given here, a more complete record being available.[12] The patient had been well up to June, 1935; at that time she began to suffer from occasional fainting spells and attacks of coma, lasting 12 to 48 hours, during which she would remain in a dazed and drowsy condition. During minor episodes, mental confusion, speech disturbances and blankness of vision were common. These attacks became more frequent until in 1937 she was having one or two a day. Attacks were very prone to occur in the early morning unless food had been taken at bedtime. A few lumps of sugar would sometimes ward off an attack. At the Worcester City Hospital, Worcester, Massachusetts, in June, 1938, the fasting blood sugar was as low as 27 mg. per cent. On a prescribed diet of high fat, low protein and carbohydrate, the patient did fairly well for a time. Then symptoms recurred, and in order to prevent an attack, feedings every 2 or 3 hours were required. On admission to the New England Deaconess Hospital on September 15, 1938, she was obese, weighing 191 pounds. Her usual weight in 1935 was believed to have been 135 pounds. General examination was negative. Fasting blood sugars varied from 30 to 50 mg. per cent. The glucose tolerance curve was characterized by a subnormal initial value, a normal course up to 3½ hours after the giving of glucose and a sharp falling away until 35 mg. per cent was reached at the end of 5 hours. A diagnosis of islet cell tumor was made. Operation was advised, and the patient went home promising to return for operation.

However, shortly after this she sustained an injury to her right ankle and remained in bed for 20 weeks. The attacks incident to hypoglycemia continued and for 2 weeks before readmission the attacks were very frequent, requiring feeding every 2½ hours. The patient did not return to the hospital until September 12, 1939, 1 year after her first admission. Examination showed extreme obesity, the weight now being 203 pounds, but no other abnormal findings were noted. Her blood sugar was low and it was unsafe to attempt fasting determinations. It was so difficult to control the hypoglycemia and the resulting stupor that it was decided to proceed at once with exploratory operation. On September 16, 1939, a laparotomy was performed by Drs. L. S. McKittrick and T. C. Pratt. This disclosed

ing cytoplasm. In such places the tumor appeared to be attempting to form ducts. (3) In a few areas the cells were polyhedral in shape with centrally placed nuclei and acidophilic cytoplasm. Occasionally these cells were binucleate. Although many cells resembled large islet cells, they failed to take any of the special stains for alpha and beta granules. None showed mitoses. In some areas strands of tumor cells were separated only by capillaries; in other areas by wide bands of hyalinized collagen. Stages of transition between these two extremes showed that collagen was laid down in increasing amounts along the walls of capillaries and later became hyalinized and relatively acellular. This hyaline material, similar to that often seen in islet cell adenomas, did not take stains for amyloid. Some hyalinized centers contained diffuse deposits of calcium. There was no relationship of the tumor nodules to the lobular structure of the liver. The general architecture and cell types were similar to those of the primary tumor. Degenerative changes were common. Clear vacuoles, relatively small and uniform in size, appeared in the cytoplasm. Some cells were packed with these vacuoles, outlined by traces of basophilic substance, and had centrally placed nuclei. Other cells were almost colorless. Foci of coagulation necrosis of tumor cells were present. Assays of insulin on the primary tumor and on the metastatic lesions in the liver were attempted, but extraction was not successful. In the surrounding liver tissue there was a striking fatty metamorphosis. While some liver cells contained a single vacuole which displaced the nucleus to one side, the predominant picture was one of cells filled with fine coalescing vacuoles in a colorless cytoplasm, leaving the nucleus centrally placed. Best's carmine stain after absolute alcohol fixation showed a moderate amount of glycogen.

The residual *pancreas* weighed 32 gm. and showed only a few minute foci of fat necrosis in the peripancreatic fat immediately adjacent to the site of operation. The pancreatic duct was patent. There was no evidence of residual tumor. The tail of the pancreas beyond the operative site showed no gross lesions. Microscopically, there were no significant changes. In the portion from the tail of the pancreas a pancreatic duct of moderate size was surrounded by large groups of islet cells, but no acini were evident. There was good differentiation between alpha and beta cells, and the latter tended to be finely vacuolated. In the remainder of the pancreas, the beta cells had few granules, but there was no evidence of hydropic degeneration or hyalinization of any islets.

The *pituitary gland* weighed 700 mg. and grossly was not remarkable. When semiserial sections were cut and stained with hematoxylin

terial. Special stains, especially Bensley's modification of Mallory's aniline blue stain [13] and Gomori's stain,[11] showed the bulk of the tumor to be made up of cells without specific granulation. However, rare, well defined beta cells were encountered, but no alpha cells. The adjacent normal pancreas provided an excellent control for the stains utilized.

The specimen from the liver measured 1.4 by 0.8 cm. and was made up of grayish pink tissue with a hard central focus. The histological structure was similar to that of the pancreatic tumor except that for the most part it was made up of central hyaline material with a few tumor cells at the periphery. No beta cells were demonstrated.

Postmortem Examination

Autopsy was performed 4 hours after death. The body was that of an obese, adult female, measuring 5 feet 2 inches in length. The anatomical diagnoses were: metastases of islet cell carcinoma in liver; thrombophlebitis of the right brachial, axillary, subclavian and internal jugular veins with complete occlusion; thrombosis with partial organization and occlusion of the superior vena cava propagating to the right auricle; massive pulmonary embolism; recent and old infarction of the right lung; marked pituitary basophilism; meningioma, 0.8 cm. in diameter.

The essential findings were: A sinus tract led from the skin surface to the region of the tail of the pancreas, but ended blindly in a smooth-walled pocket which did not communicate with the pancreas and contained amorphous material.

The *liver* weighed 1510 gm. Externally and on the cut surface it was studded with many firm, white, glistening, well demarcated nodules varying from 2 to 3 cm. in diameter. Those on the surface tended to be umbilicated. Many contained firm, hard, yellow centers. The tumor replaced one-third to one-half of the liver substance. Uninvolved portions of the liver were firm and reddish brown.

Microscopically, the liver nodules varied greatly in size and cellular content. The manner of growth resembled an islet on a large scale. There were three types of cells: (1) The cell type found most frequently was tall columnar, and such cells were aligned in long ribbonlike strands. The cytoplasm was generally acidophilic, but some foci showed a small amount of basophilic, finely granular material in one end of the cell. In other foci the spaces between the cells were filled with strands of amorphous material with a basophilic tinge suggesting thin mucus. (2) In other parts, tall, regular columnar cells formed tubes with basement membranes. The nucleus was at the membrane end of such cells, while the inner two-thirds was composed of clear, mucus-contain-

TABLE I

Summary of Nine Cases of Islet Cell Tumors with Metastases

Case no.	Author	Age	Weight increase (pounds)	Total length of symptoms (months)	Operation	Result	Assay of insulin	Autopsy
1	Wilder, Allan, Power and Robertson[2]	40	25	21	Exploratory: inoperable carcinoma	Died postoperatively	Present in liver nodule	Metastases in liver, lymph nodes and mesentery; pituitary not mentioned
2	Judd, Faust and Dixon[3]	18	30	4.5	Exploratory: tumor of pancreas and multiple metastases in liver; biopsy of liver nodule	Died postoperatively	Not done	Not done
3	Bickel, Mozer and Junet[4]	56	Not mentioned	7	None	Died without operation	Present in primary growth; liver nodule negative	Metastases in liver, peritoneum and epicardium; interstitial pancreatitis; pituitary not mentioned
4	Cragg, Power and Lindem[5]	51	Not mentioned	9	Exploratory: biopsy of liver	Died postoperatively	Present in liver nodule	Metastases in liver and lymph nodes; diffuse tumor in pancreas; pituitary not examined
5	Joachim and Banowitch[6]	31	Not mentioned; tendency towards obesity	2	Resection of spleen and portion of tumor in tail of pancreas and a lymph node showing metastases; liver apparently negative	Died postoperatively	Not done	Not done
6	Flinn, Beatty, Ginsberg and Hemsath[8]	45	Not mentioned	5	None	Died without operation	Not carried out successfully	Tumor in head of pancreas; metastases in liver and lymph nodes; pituitary "grossly" normal
7	Jacobsen[9]	36	Not mentioned	12–16	Exploratory: inoperable carcinoma in pancreas; metastases in liver	Died postoperatively	Not done	Tumor in pancreas eroded into duodenum; metastases in liver; pituitary not mentioned
8	Cannon (personal communication) See also references 10 and 11	36	16	12	Exploratory: inoperable carcinoma in pancreas; metastases in liver	Died postoperatively	Inconclusive	Metastases in liver, lymph nodes, adrenals, spine, lungs and pleura; pituitary not examined
9	Gray	48	65	48	Resection of pancreatic tumor; biopsy of metastatic lesion in liver	Died postoperatively	Not carried out satisfactorily	Metastases in liver; marked basophilism of pituitary gland

and eosin, the central half of the anterior lobe was seen to be composed of massed acidophilic cells surrounded by a narrow border of mixed eosinophilic, basophilic and chromophobe cells. The peripheral half showed a marked predominance of basophilic cells, a scattering of eosinophilic cells and a reduction in chromophobe cells. The basophilic cells tended towards an adenomatous arrangement. The majority of these cells contained deeply stained granules; others, light blue-staining granules. Vacuoles in the cytoplasm were prominent and numerous. Throughout the lobe were many small spaces surrounded by mixed cells and filled with colloid material which was usually acidophilic. A few, however, were lined entirely by basophilic cells and filled with basophilic colloid. The pars intermedia was marked by large, elongated acini lined by flat, slightly basophilic epithelial cells and filled with abundant acidophilic colloid. The posterior lobe was structurally normal, but heavily infiltrated anteriorly by basophilic cells.

Comment

Clinical Observations

The eight patients previously reported (Table I), as well as the patient herein reported, died, most of them within 1 year of first symptoms whether or not operation had been done. In case no. 9, the long duration, over 4 years, is interesting. The progressive nature of the symptoms was characteristic in the histories of all nine patients in that hypoglycemic attacks eventually occurred and were finally difficult to control even when a constant feeding regime was carried out.

The obesity in case no. 9 was extreme. Weight gain was mentioned in only three of the cases (nos. 1, 2 and 8) though it has been commonly stated in reports of islet cell adenomas.

The development of transitory hyperglycemia and glycosuria, indistinguishable from diabetes mellitus, in the postoperative period occurred only in case no. 9. It is interesting also that this is the single instance where the primary tumor was completely removed. One could speculate that the metastatic lesions were non-functioning, but such speculation would necessitate the acceptance of the theory that the pancreas also was not producing sufficient insulin to maintain the blood sugar within normal limits and the urine sugar-free. It should be noted that the hyperglycemia and glycosuria occurred after the administration of intravenous glucose had been stopped. Furthermore, the patient had normal blood-sugar determinations and no symptoms of hypoglycemic shock during the 10-day period prior to death, and no insulin was given. The picture was further complicated by infection. Hence no conclusions are drawn.

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Pathological Observations

The cells of both the primary and the metastatic lesions bore a striking histological resemblance to islet cells. The fact that beta cells were demonstrated in the pancreatic tumor, but not in the metastatic lesions, is interesting, but in the absence of satisfactory assays of insulin, no definite correlation with the clinical picture may be drawn. Indeed, it has been stressed by Campbell, Graham, and Robinson [14] that, when the amount of tumor is known and when careful assays of insulin have been carried out and specific islet cell types identified, the information may not be closely correlated with the clinical picture.

In eight cases metastases were localized in the liver, mesenteric lymph nodes and epicardium. In case no. 8, however, generalized metastases were found and the widespread nature of the metastases leaves no doubt that they were carried in the blood stream.

The basophilism of the pituitary gland in case no. 9 is striking. The massed eosinophils, while prominent, are probably not significant. No histological changes in the pituitary glands were recorded in the other eight cases. Increase in basophilic cells has been described in cases of islet cell adenoma with hypoglycemia. Friedman's [15] two cases showed marked basophilic adenomatous hyperplasia of the anterior lobe and marked obesity. He reviewed all the other cases to date in which pituitary lesions associated with islet cell tumors were described. Many observers [15-17] had noted in those suffering from obesity larger numbers of basophils as well as more basophilic and transitional cell adenomas than in non-obese persons.

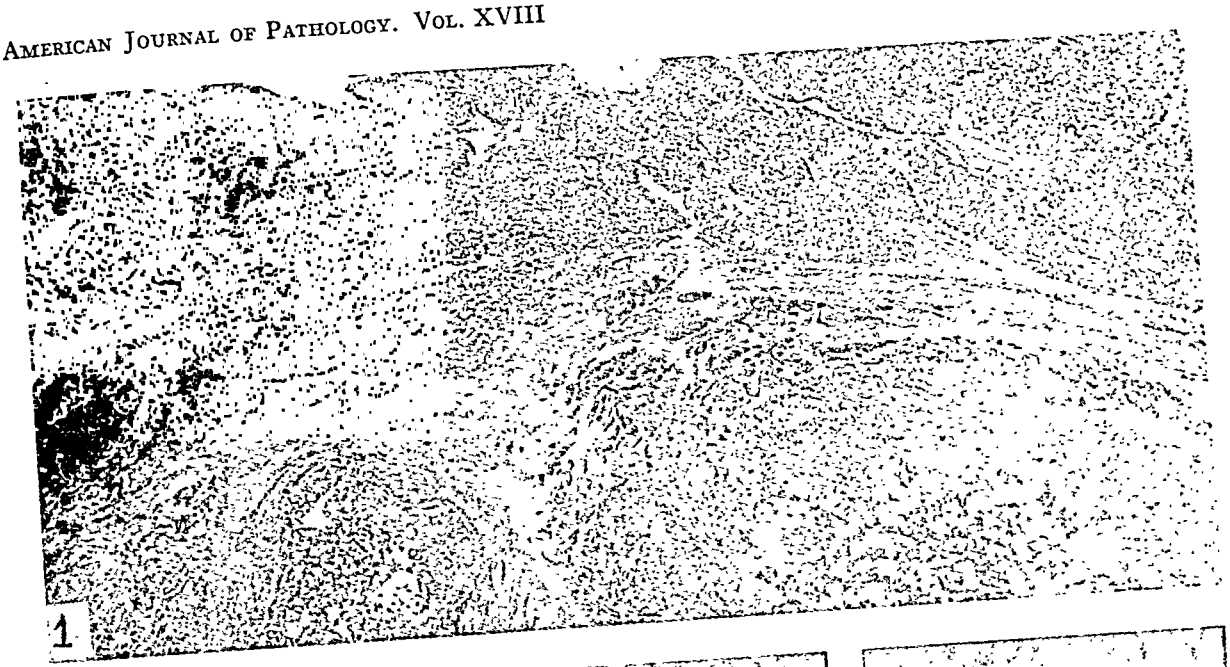
Summary

A case of functioning islet cell carcinoma of the pancreas with metastases in the liver is reported and compared with eight other proved islet cell carcinomas with metastases. The development of a transient hyperglycemia and glycosuria in the postoperative period following removal of the primary tumor is noted. Beta cells were demonstrated in the primary tumor, but not in the metastatic lesions. Pituitary basophilism also was found.

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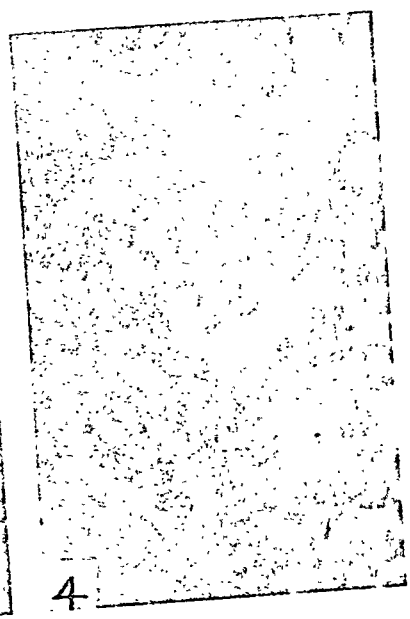
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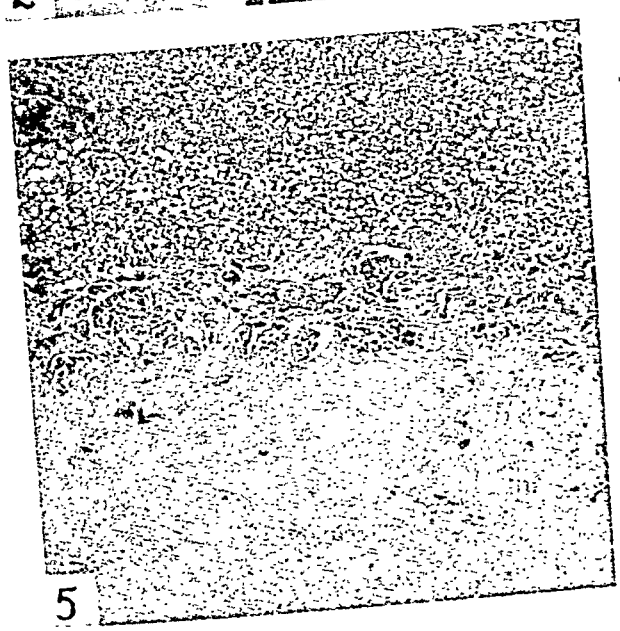
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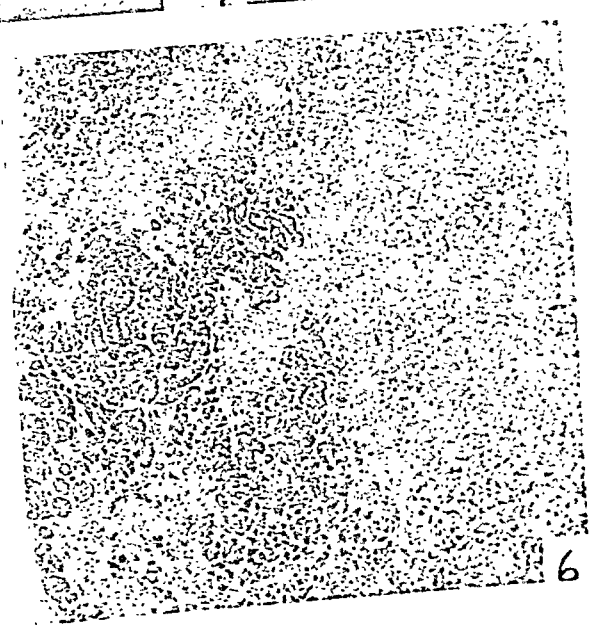












Functioning Islet Cell Carcinoma

DESCRIPTION OF PLATE

PLATE 100

FIG. 1. General topography of primary tumor showing invasion of capsule. × 17.

FIG. 2. Primary tumor. The cells are arranged in islet-like strands. × 250.

FIG. 3. Primary tumor. The cells tend to form ductlike structures. × 250.

FIG. 4. Primary tumor. The cells are grouped in alveolar-like masses. × 250.

FIG. 5. Tumor in liver. Marked hyalinization. The surrounding liver tissue shows fatty metamorphosis. × 40.

FIG. 6. Pituitary gland. Deep-staining basophilic cells arranged in adenomatous masses. ×23.

GLYCOGEN INFILTRATION OF THE LIVER CELL NUCLEI *

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One of the most important functions of the liver is the synthesis of glycogen, a storage form of carbohydrate. This is a labile substance readily convertible into glucose, and although it is present in abundance in the cytoplasm of the liver cells after the ingestion of carbohydrates, it decreases or even disappears during fasting or starvation. On the other hand, glycogen is not found normally in the nuclei of the liver cells at any time. This report is devoted chiefly to a study of the pathological infiltration of liver-cell nuclei by glycogen, but during the course of this study it was possible to confirm certain earlier observations regarding the normal storage of glycogen in liver cells. These observations will first be reviewed briefly because the facts established by histological studies of glycogen deposition in the liver seem not to be very widely known.

Fixation and Staining

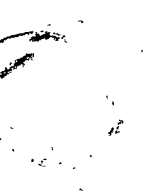
Glycogen is the only carbohydrate that can be studied histologically, being stained a brownish red colour by iodine and crimson by the more widely used Best's carmine stain. It is water-soluble and in order to retain it in the tissue for examination, water and predominantly aqueous solutions must be avoided before and after fixation. Ordinarily this end is achieved by fixing blocks of tissue in absolute alcohol and embedding them in celloidin.[1] However, in this study the following technic was employed:

Thin blocks of liver were fixed in a solution made up of 9 parts of absolute alcohol and 1 part of 40 per cent formaldehyde.† After fixation for 24 hours or longer the blocks of tissue were dehydrated in the usual manner, beginning with 95 per cent alcohol, and embedded in paraffin. Sections were cut at a thickness of 6 μ and mounted on ordinary glass slides. The paraffin was removed by xylol and the sections were then coated with a thin film of celloidin by pouring on a few drops of a 0.3 per cent solution of celloidin in equal parts of absolute alcohol and ether. Then the sections were run down to water following which the ordinary Best's carmine method of staining[1] was applied, the sections were freed from celloidin by acetone, dehydrated and mounted in Canada balsam.

By this method, thin sections can be readily obtained and handling of the sections is simplified by the fact that they are mounted on glass

* Received for publication, September 22, 1941.

† Dr. Shields Warren, in a personal communication, stated that he has found Carnoy's fluid a more satisfactory fixative than either absolute alcohol or the formaldehyde and absolute alcohol solution used in this study.



and constant when large amounts of glycogen are present, lesser amounts of glycogen may cause scarcely any discernible change in the appearance of the cytoplasm in paraffin sections. Consequently, in order to exclude or detect the presence of small quantities of glycogen in the cytoplasm of liver cells, resort must be had to the Best's carmine stain. Thus the liver-cell cytoplasm may or may not contain glycogen under normal conditions.

Intranuclear Glycogen

The liver-cell nuclei normally do not contain glycogen. However, in 1883 Ehrlich [4] noted large glycogen-filled vacuoles in the liver cells in cases of diabetes mellitus. Later these vacuoles were shown to be the nuclei and since then it has been generally recognized that glycogen often infiltrates the liver-cell nuclei in diabetic persons. It has also been known for a long time, though not widely recognized, that glycogen frequently can be demonstrated in the liver-cell nuclei in non-diabetic persons.[5] For this reason Warren [5] believes that the mere presence of glycogen in the nuclei should not be regarded as a particularly significant lesion in diabetes. He does emphasize, however, that there is a significant reciprocal relation between the amounts of intranuclear and of intracytoplasmic glycogen. He also presents evidence to show that insulin promotes the deposit of glycogen in the liver-cell cytoplasm. In other words, he suggests that in the diabetic patient there tends to be an absence of glycogen-containing nuclei in the liver if he is well treated with insulin, while in uncontrolled cases glycogen-containing liver-cell nuclei are likely to be present in abundance.

In ordinary paraffin sections of the liver from cases of uncontrolled diabetes, one frequently sees peculiar vacuolated, empty-appearing nuclei in large numbers, situated in the peripheral cells of the liver lobules. It is usually assumed that these nuclei are the glycogen-containing ones. Similar vacuolated nuclei in similar distribution are also frequently seen in the liver in non-diabetic cases. In our earlier autopsy records this vacuolation of liver-cell nuclei has often been described as though representing some form of degenerative change, but almost never has there been any speculation as to the nature of the abnormality. It was the purpose of the study here reported to determine whether these vacuolated nuclei in the liver cells of diabetic patients are actually the ones which contain glycogen; whether this peculiar vacuolation of liver-cell nuclei is always associated with the intranuclear accumulation of glycogen and whether the presence of any considerable quantity of glycogen in the nucleus always produces

slides at the outset. Altogether the procedure outlined is much quicker and easier than the usual celloidin method and gives equally reliable results.

Intracytoplasmic Glycogen

Best's carmine stain reveals the glycogen, if present, as small or large crimson granules distributed through the cytoplasm of the liver cells. Tissue culture studies,[2] however, have shown the glycogen to be actually in solution in the cytoplasm of the living cells and therefore its granular appearance in stained fixed tissue must be regarded as a constant fixation artefact. In livers containing large amounts of glycogen it is found to be fairly evenly distributed throughout the liver lobule, but as it is released from the liver it disappears first from the central cells of the lobule. Consequently, when present at all it is generally found to be most abundant in the periportal portions of the lobule. A tendency for the glycogen granules to be concentrated in the same side of each cell is due to the alcohol fixative having driven the glycogen ahead of it as it penetrated the tissues.[1]

In a large series of autopsy cases, in which the livers were stained for glycogen in the present study, a correlation could generally be shown between the amount of glycogen present and the elapsed time since food intake before death. In cases where death is unexpected and sudden, as from physical trauma or pulmonary embolism, glycogen is generally abundant in the liver. On the other hand, when there is a long agonal period of starvation, glycogen is generally scanty or absent.

The interval between death and the time of fixation of the liver up to 8 or 10 hours does not materially affect the appearance and quantity of glycogen in the liver, as estimated in sections stained with Best's carmine. Once the tissue is fixed in the alcohol-formaldehyde solution the glycogen apparently will remain unchanged for indefinite periods. Even after several years formaldehyde-fixed tissues will sometimes show the glycogen granules fairly well.

It is generally known [3] that the presence of large amounts of glycogen in the liver-cell cytoplasm will cause a characteristic appearance in ordinary paraffin sections stained with haematoxylin and eosin (Fig. 1). The cytoplasm, instead of presenting its more usual homogeneous character, becomes pale and fluffy in appearance. This appearance is sometimes mistaken for a degenerative change and it should be emphasized that it merely denotes the normal presence of glycogen which, of course, actually has been dissolved out during the fixation and staining process. Although this fluffy appearance is characteristic

Without exception it was found that in those cases in which the peculiar vacuolated liver-cell nuclei were present in the routine sections, a roughly equivalent number of nuclei could be shown to contain glycogen in the liver sections stained with Best's carmine. The closeness of the numerical correspondence between the abnormal nuclei in the two types of preparation is indicated by the fact that in no instance was there a major disagreement between the grades which had been assigned to represent the estimated numbers of abnormal nuclei. Actually, the nuclei in question were more readily detected in sections stained with haematoxylin and eosin than in those stained for glycogen. That the empty-appearing nuclei seen in the former type of preparation really corresponded with those which contained glycogen in the sections stained with Best's carmine could scarcely be doubted after comparison of the sections under the microscope. Apart from the presence of one or more droplets of crimson-stained intranuclear glycogen in the sections stained to demonstrate its presence, the morphology of the abnormal nuclei was identical in the two types of histological preparation (Figs. 3 and 4). There was no instance of glycogen infiltration of liver-cell nuclei in which the peculiar nuclear vacuolation was lacking. Among the 160 cases studied, 94 were completely negative, that is, the liver-cell nuclei neither contained glycogen nor showed any sort of vacuolation. In 66 cases, or 41 per cent, small or large numbers of liver-cell nuclei were vacuolated and contained glycogen; in 24 of these cases, or 15 per cent of the total, considerable numbers of nuclei were found to be vacuolated by the presence of glycogen, and the abnormal nuclei were present in sufficient abundance that the sections had been assigned grades of 3 or 4.

The positive and negative evidence accumulated by the study of this first group of cases was supplemented by further evidence on the positive side obtained from the study of an additional group of 155 autopsy cases which were dealt with in the following manner. Blocks of liver tissue from each case were preserved in the alcohol-formaldehyde fixative in addition to the blocks of tissue taken routinely. The routine paraffin sections of liver from these same cases were studied and graded, as before, according to the numbers of abnormal vacuolated nuclei. In each case in which the peculiar vacuolated liver-cell nuclei were found in the routine sections, further histological sections were prepared from the specially fixed liver tissue and stained for glycogen. Again, in every instance, the peculiar empty-appearing nuclei seen in the routine liver sections stained with haematoxylin and eosin proved, in the sections stained for glycogen, to be glycogen-containing. In this group of 155 cases, 57 cases, or 37 per cent, showed the pres-

vacuolation visible in paraffin sections. In short, can this type of vacuolation of liver-cell nuclei be depended upon as a reliable indication of the presence of intranuclear glycogen?

The appearance, if not the nature, of these peculiar vacuolated liver-cell nuclei, as seen in paraffin sections stained with haematoxylin and eosin is probably familiar to all who see routine autopsy material (Figs. 2 and 3). They are nearly always confined to the liver cells at the periphery of the lobules about the portal areas, although when present in large numbers they may approach the central part of the lobules. Thus, they occur predominantly in the cells which, during depletion of liver glycogen, are the last to give up their cytoplasmic stores of this carbohydrate. In their most striking form these vacuolated nuclei are very large, measuring up to 25 μ or more in diameter; they are generally somewhat irregular in outline, even crescentic or crenated. The nuclear chromatin and the nucleolus, if it is visible, are pushed to the periphery of the nucleus so that the nuclear membrane appears to have been overtraced with deep purple dye. The central part of the nucleus either appears completely empty and unstained, or it presents a pale, slightly opaque, violet or gray homogeneous appearance. The empty-appearing nuclei are indeed just that, for the diameter of the swollen nuclei being greater than the thickness of the histological section, the tops and bottoms of these nuclei have been cut off and one is in reality looking through an empty hole in the section. If histological sections of increasing thickness are prepared, the holes finally disappear in the thicker sections and only the nuclei with gray homogeneous centres remain. The latter, therefore, represent empty nuclei in which the nuclear membrane is intact over the upper or lower surface. By focusing up or down, this part of the nuclear wall is often clearly revealed.

In order to study the relation of this characteristic vacuolation of liver-cell nuclei to the intranuclear accumulation of glycogen, blocks of liver from 160 consecutive autopsy cases were fixed and stained for glycogen by the modification of the Best technic already described. Only newborn infants were excluded from this series, since preliminary studies had revealed no instance of vacuolation of the liver-cell nuclei in the newborn. The routine paraffin sections of the livers from the same 160 cases were examined, as well as the special sections stained for glycogen, but the examination of the two types of preparation was carried out independently and in each instance the specimen was graded as 0, 1, 2, 3 or 4 according to the examiner's estimate of the abundance of the peculiar vacuolated nuclei, described above, in the sections stained with haematoxylin and eosin, or the abundance of glycogen-containing nuclei in the sections stained with Best's carmine.

and note taken of the principal anatomical findings, as well as other factors of possible importance such as state of nutrition and degree of arteriosclerosis. The histological sections of the liver in each case were reëxamined for lesions other than glycogen infiltration of the nuclei.

Clinical Features

It became apparent almost from the beginning of this analysis that many different kinds of cases were represented. Of the 140 cases, 32, or 23 per cent, were cases of diabetes mellitus. Since it is well established that glycogen infiltration of the liver-cell nuclei is common in diabetes, it was important to exclude the possibility of diabetes in the remaining supposedly non-diabetic cases. In deciding whether or not any given case was one of diabetes, the chief determining factor had to be whether or not such a diagnosis had been made clinically, but cases were considered as being cases of diabetes if there was any hint at all that they might have been. The figure of 23 per cent is therefore a maximum figure. On the other hand, in the cases considered to be non-diabetic, urinalysis showed the absence of sugar; blood sugar, when determined, was within normal limits, and nowhere in the case histories was the possibility of diabetes entertained. Perhaps if all the facts had been known, some of these cases might have proved to be cases of diabetes, but undoubtedly the great majority of the 108 cases considered to be non-diabetic were truly non-diabetic.

In only one case of diabetes was death attributed to uncomplicated diabetic coma, and for further consideration of the cases, diabetes, *per se,* was regarded simply as one of the many possible factors which might be responsible for glycogen infiltration of the liver-cell nuclei. The diabetic and non-diabetic cases were, therefore, considered together in the analysis of the cases for other factors of possible importance. This study did not reveal any other factor of constant causal significance nor any factor which was common to all cases, but the following features, briefly recorded, seemed noteworthy.

Among the 140 cases analyzed, the ages varied from 11 months to 82 years with an average of 53 years. It was apparent that the age of the patients was related to the disease from which they died and that there was no correlation between age and the presence or absence of glycogen in the liver-cell nuclei. Males constituted 54 per cent of the cases and females 46 per cent, a proportion between the sexes which does not differ greatly from that encountered within recent years among the cases coming to autopsy in this department.

No correlation could be established between the duration of illness and the presence of glycogen-containing nuclei in the liver. The short-

ence of vacuolated glycogen-containing liver-cell nuclei, and in 20 cases, or 13 per cent of the total, these abnormal nuclei were present in large numbers. This group of cases, combined with the first group studied, makes up a total of 315 cases. Vacuolated glycogen-containing liver-cell nuclei were demonstrated in 123 of these cases, or in 39 per cent, while in 44 cases, or in 14 per cent of the total, the abnormal nuclei were present in large numbers.

These findings, showing as they do a perfect correlation between glycogen infiltration and vacuolation of the liver-cell nuclei, were deemed sufficient to justify the conclusion that glycogen infiltration of the nuclei of the liver cells regularly produces a peculiar vacuolation of these nuclei which is easily recognizable in paraffin sections stained by routine methods. Moreover, these results indicate that this peculiar vacuolation does not occur except in association with infiltration of the nucleus by glycogen. It can therefore be stated that the characteristic vacuolation of liver-cell nuclei, already described, can be depended upon as a reliable indication of the presence of intranuclear glycogen.

Analysis of Cases of Intranuclear Glycogen Deposit

In the hope of determining the causes of glycogen infiltration of the liver-cell nuclei, the clinical and autopsy records were studied carefully in a series of cases in which large numbers of glycogen-containing nuclei were present in the liver. Only 44 such cases had been found among the 315 cases, as has been described in the preceding section of this report. However, the conclusions drawn from that study were made use of in the selection of additional cases of marked glycogen infiltration of the liver-cell nuclei. The routine sections, stained by haematoxylin and eosin, of the livers from 932 consecutive cases from the autopsy files, were examined with a view to selecting those cases in which the presence of many characteristically vacuolated liver-cell nuclei gave clear indication of glycogen infiltration of a degree equivalent to that found in the original 44 cases. This search yielded 96 additional cases, bringing the total to 140 cases available for analysis.

The hospital records of these 140 cases were examined. Among the features, other than the clinical diagnosis, of which particular note was taken were such factors as age, sex, total duration of illness, duration of severe illness, duration of critical illness, interval between the last intake of food and death, the use and quantity of intravenous glucose during the final illness, history of diabetes mellitus, the urinary findings, blood chemistry, blood pressures, blood counts, presence of fever, duration of fever, medication and symptoms such as dyspnoea and cyanosis. The autopsy protocols of these cases were likewise reviewed

There were 25 cases in which the outstanding lesion was a neoplasm. Among these were carcinomata of the breast, tongue, thyroid, larynx, bronchus, stomach, gallbladder, rectum and cervix. There was one lymphosarcoma, a chromaffin tumour of the adrenal, 2 cases of chromophobe adenoma of the pituitary, a suprasellar craniopharyngioma, a colloid cyst of the third ventricle and a glioblastoma. In many instances, extensive tumour metastases and necrosis with secondary infection were associated findings.

There were three cases of tuberculous meningitis, in two of which there was also a generalized miliary tuberculosis. All of these showed clinically a marked increase in intracranial pressure, and large tuberculomata of the brain were demonstrated in each case at autopsy.

One small but interesting group was made up of 4 cases of diphtheria in young children. In each, the diagnosis of diphtheria was established both clinically and pathologically. It is interesting to note that in 1900, Councilman, Mallory and Pearce,[6] in an extensive study of the pathology of diphtheria, described a peculiar vacuolation of the liver-cell nuclei as a frequent but inconstant finding. They regarded the lesion as a rare form of degeneration of the affected nuclei, but they realized that it was not peculiar to diphtheria for they noted that many such nuclei had been observed in a case of leukemia. Examination of the sections of liver from other cases of diphtheria in the autopsy files of this department revealed glycogen-containing nuclei in large numbers in at least one-half of the cases, but they were not a constant finding.

The remaining 14 cases could not easily be grouped or else were single examples of disease entities. There was 1 case of myasthenia gravis and 1 in which the patient died of pulmonary embolism 14 days after pregnancy had been terminated because of extreme toxemia.

A regrouping of some of the cases was prompted by consideration of the four cases of diphtheria, all of which were brought to hospital late in the illness and showed clinically symptoms and signs of suffocation. In six other cases as well, cyanosis and signs of strangulation were outstanding clinical features. One of these was a case of carcinoma of the tongue in which death finally occurred from respiratory obstruction. The same mode of death was observed in a case of carcinoma of the larynx, in another in which a large collar-like carcinoma of the thyroid compressed the trachea, and again in a case of extensive tumour infiltration of the mediastinum from a primary carcinoma of the breast. Another similar case was that of a patient with uncontrollable asthma who finally died with extreme respiratory embarrassment. The sixth case of this type was one in which the patient, a man 69 years old, dying of pneumonia, was found at autopsy to have an extremely nar-

est interval between the onset of disease and death in a person previously in apparently good health was in a child, 2 years of age, who had died of diphtheria on the sixth day of illness. No correlation was observed with food intake nor with the intravenous administration of glucose. Likewise the medication employed in different cases presented no features common to all cases.

The blood pressure was moderately or extremely elevated in 40 per cent of the cases. There were no consistent findings on examination of the blood. However, the white blood cell count was elevated in many instances.

Associated Anatomical Lesions

The anatomical diagnoses recorded in the 140 cases covered a wide range, but it is noteworthy that there did not occur in the series a single case of uncomplicated traumatic death. This merely serves to emphasize the fact that glycogen infiltration of the liver-cell nuclei does not occur under normal conditions. The body was described as of average nutrition in 55 per cent of the cases, as obese in 25 per cent and as cachectic in 20 per cent.

Because of the variety of lesions, the cases could be grouped only in the most arbitrary manner according to the nature of the principal lesions which recurred most frequently. There was sometimes overlapping of certain features among cases placed in separate groups.

In 44 cases, or 31 per cent of the total, the outstanding feature at autopsy was the presence of marked inflammatory lesions usually accompanied by more or less extensive necrosis of tissue. Typical of such lesions were instances of multiple pulmonary abscesses, bilateral pyonephrosis, actinomycosis, gangrene of the lower extremities with septicaemia, phlegmon of the abdominal wall and extensive necrosis and secondary infection of bladder and vagina associated with carcinoma of the cervix. The locations of such lesions were most varied. Thirteen of the 32 cases of diabetes mellitus fell into this group.

In 70 per cent of the cases there was moderate or marked arteriosclerosis. There were 31 cases in which the principal diagnosis was some cardiac lesion leading to heart failure. In 14 of these cases there was coronary thrombosis and myocardial infarction. In 19 cases the principal lesion was a cerebrovascular one, either cerebral softening or haemorrhage. In combining these two latter groups, totaling 50 cases, or 36 per cent of the whole series, it is interesting to note that there were 5 cases in which there was a necrotizing arteriolosclerosis. Among these cases, too, necrosis of tissue was present in 33 instances in the form of myocardial infarction or encephalomalacia. Fourteen of the 32 cases of diabetes fell into this combined group of 50 cases.

There were 25 cases in which the outstanding lesion was a neoplasm. Among these were carcinomata of the breast, tongue, thyroid, larynx, bronchus, stomach, gallbladder, rectum and cervix. There was one lymphosarcoma, a chromaffin tumour of the adrenal, 2 cases of chromophobe adenoma of the pituitary, a suprasellar craniopharyngioma, a colloid cyst of the third ventricle and a glioblastoma. In many instances, extensive tumour metastases and necrosis with secondary infection were associated findings.

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A regrouping of some of the cases was prompted by consideration of the four cases of diphtheria, all of which were brought to hospital late in the illness and showed clinically symptoms and signs of suffocation. In six other cases as well, cyanosis and signs of strangulation were outstanding clinical features. One of these was a case of carcinoma of the tongue in which death finally occurred from respiratory obstruction. The same mode of death was observed in a case of carcinoma of the larynx, in another in which a large collar-like carcinoma of the thyroid compressed the trachea, and again in a case of extensive tumour infiltration of the mediastinum from a primary carcinoma of the breast. Another similar case was that of a patient with uncontrollable asthma who finally died with extreme respiratory embarrassment. The sixth case of this type was one in which the patient, a man 69 years old, dying of pneumonia, was found at autopsy to have an extremely nar-

est interval between the onset of disease and death in a person previously in apparently good health was in a child, 2 years of age, who had died of diphtheria on the sixth day of illness. No correlation was observed with food intake nor with the intravenous administration of glucose. Likewise the medication employed in different cases presented no features common to all cases.

The blood pressure was moderately or extremely elevated in 40 per cent of the cases. There were no consistent findings on examination of the blood. However, the white blood cell count was elevated in many instances.

Associated Anatomical Lesions

The anatomical diagnoses recorded in the 140 cases covered a wide range, but it is noteworthy that there did not occur in the series a single case of uncomplicated traumatic death. This merely serves to emphasize the fact that glycogen infiltration of the liver-cell nuclei does not occur under normal conditions. The body was described as of average nutrition in 55 per cent of the cases, as obese in 25 per cent and as cachectic in 20 per cent.

Because of the variety of lesions, the cases could be grouped only in the most arbitrary manner according to the nature of the principal lesions which recurred most frequently. There was sometimes overlapping of certain features among cases placed in separate groups.

In 44 cases, or 31 per cent of the total, the outstanding feature at autopsy was the presence of marked inflammatory lesions usually accompanied by more or less extensive necrosis of tissue. Typical of such lesions were instances of multiple pulmonary abscesses, bilateral pyonephrosis, actinomycosis, gangrene of the lower extremities with septicaemia, phlegmon of the abdominal wall and extensive necrosis and secondary infection of bladder and vagina associated with carcinoma of the cervix. The locations of such lesions were most varied. Thirteen of the 32 cases of diabetes mellitus fell into this group.

In 70 per cent of the cases there was moderate or marked arteriosclerosis. There were 31 cases in which the principal diagnosis was some cardiac lesion leading to heart failure. In 14 of these cases there was coronary thrombosis and myocardial infarction. In 19 cases the principal lesion was a cerebrovascular one, either cerebral softening or haemorrhage. In combining these two latter groups, totaling 50 cases, or 36 per cent of the whole series, it is interesting to note that there were 5 cases in which there was a necrotizing arteriolosclerosis. Among these cases, too, necrosis of tissue was present in 33 instances in the form of myocardial infarction or encephalomalacia. Fourteen of the 32 cases of diabetes fell into this combined group of 50 cases.

gen staining of sections of the liver, it is demonstrated that such abnormal, vacuolated nuclei invariably contain glycogen. Conversely, it is shown that glycogen infiltration of the liver-cell nuclei regularly produces the characteristic vacuolation of these nuclei which is easily recognizable in paraffin sections stained by routine methods. Such vacuolation of the liver-cell nuclei can, therefore, be depended upon as a reliable indication of the presence of intranuclear glycogen.

In a series of 315 unselected autopsy cases from which only newborn infants were excluded, glycogen infiltration of the liver-cell nuclei was demonstrated by glycogen staining in 123 cases, or in 39 per cent of the total. The abnormal glycogen-containing nuclei were present in large numbers in 44 cases, or in 14 per cent of the whole series.

In a series of 140 cases in which large numbers of liver-cell nuclei contained glycogen, the clinical data and autopsy findings were analyzed in the hope of determining the causes of glycogen infiltration of the liver-cell nuclei. Cases of diabetes mellitus made up 23 per cent of the series. The other cases, as well as the diabetic cases, showed a great variety of lesions. The analysis of clinical and autopsy data failed to reveal any factor of constant causal significance other than the presence of uncontrolled diabetes mellitus. Nevertheless, many of the features of the cases studied seemed worthy of note and these have been briefly recorded. The ultimate cause of glycogen infiltration of the liver-cell nuclei remains unknown.

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row, sabre-shaped, calcified trachea. Dyspnoea and cyanosis were prominent in many of the cardiovascular cases also and in other cases with extensive pulmonary lesions such as abscesses, bronchial carcinoma or tuberculous cavitation. These could possibly be placed in the same group, but in them respiratory embarrassment was not so extreme nor could it be so readily evaluated from anatomical findings.

The four cases of tumour centering around the pituitary or floor of the third ventricle, as well as the one case of cerebral glioma and the three cases of tuberculous meningitis with markedly increased intracranial pressures, were thought possibly to form a significant group. However, examination of the histological sections of the liver in additional selected cases of this kind, in search of glycogen-containing nuclei, failed to reveal any constant correlation.

Apart from glycogen infiltration of the liver-cell nuclei, the liver itself in many cases showed no pathological alterations and in the remaining cases all manner of changes were observed. Chronic passive congestion of varying degree was a frequent occurrence, while other findings included fatty metamorphosis, periportal hepatitis and secondary carcinoma. Cirrhosis of the liver was present in only one case.

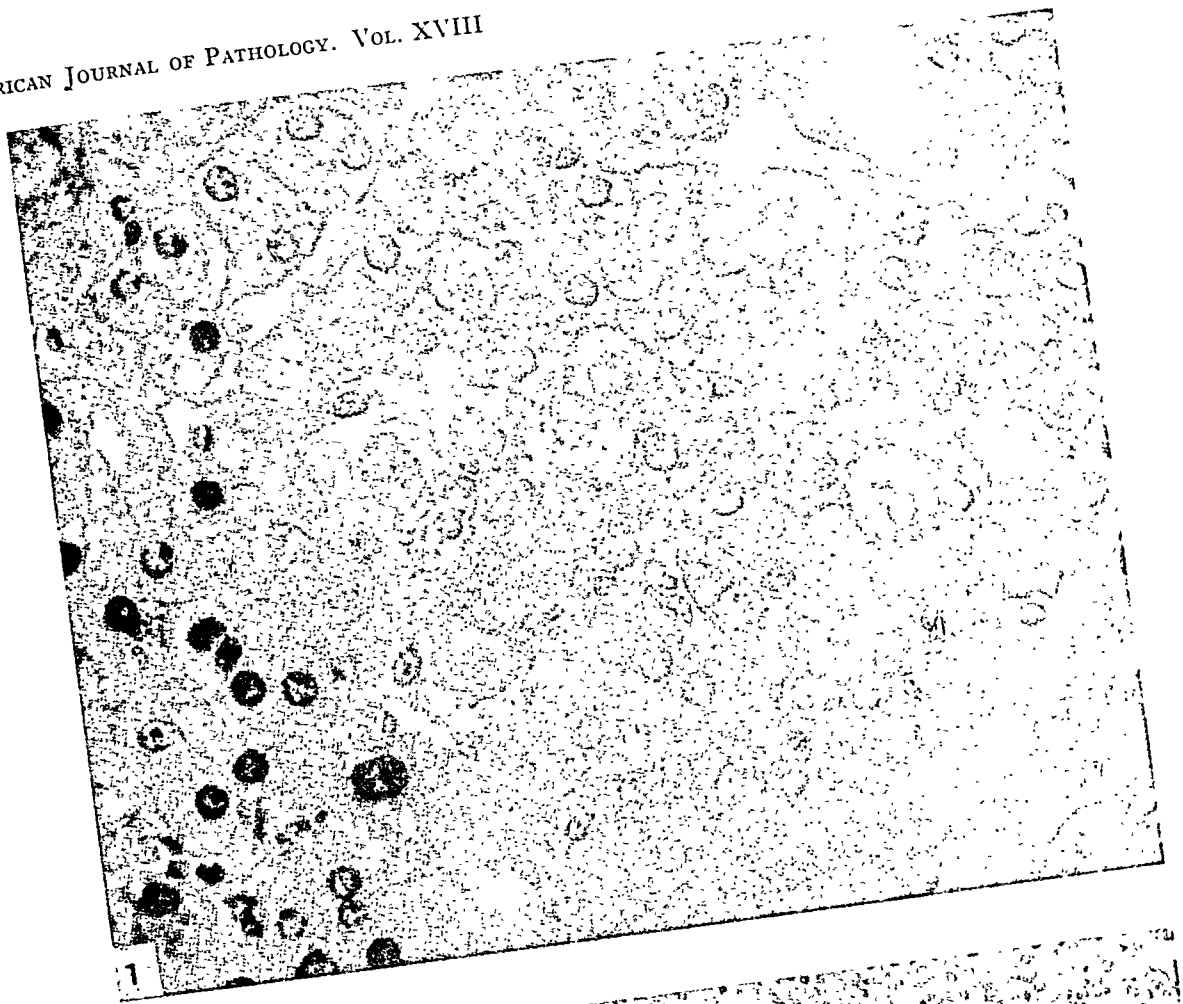
Thus, the analysis of 140 cases of marked glycogen infiltration of the liver-cell nuclei demonstrated only that this abnormality occurs as an accompaniment of a considerable variety of diseases. The more frequently associated conditions were diabetes mellitus; acute suppurative inflammatory processes; neoplasms; inflammatory, arteriosclerotic and neoplastic lesions of various kinds in which necrosis of tissue was a prominent feature, and lesions causing respiratory embarrassment with relative anoxia. The analysis provided no real clue as to the ultimate cause of glycogen infiltration of the liver-cell nuclei.

The grouping of the abnormal glycogen-containing nuclei in the periportal regions of the liver lobules suggests the possibility that some factor referable to the blood supply may be responsible for the occurrence of glycogen infiltration of the nuclei in these areas rather than elsewhere in the liver lobules. A significant relationship is also suggested by the fact that the periportal liver cells in which the abnormal nuclear accumulation of glycogen occurs are those which normally, during depletion of liver glycogen, are the last to give up their cytoplasmic glycogen stores.

Summary and Conclusions

Peculiar empty-appearing or vacuolated liver-cell nuclei with a peripheral arrangement of the chromatin are described as occurring not infrequently in paraffin sections of the liver from routine autopsy cases when stained by haematoxylin and eosin. By means of parallel glyco-

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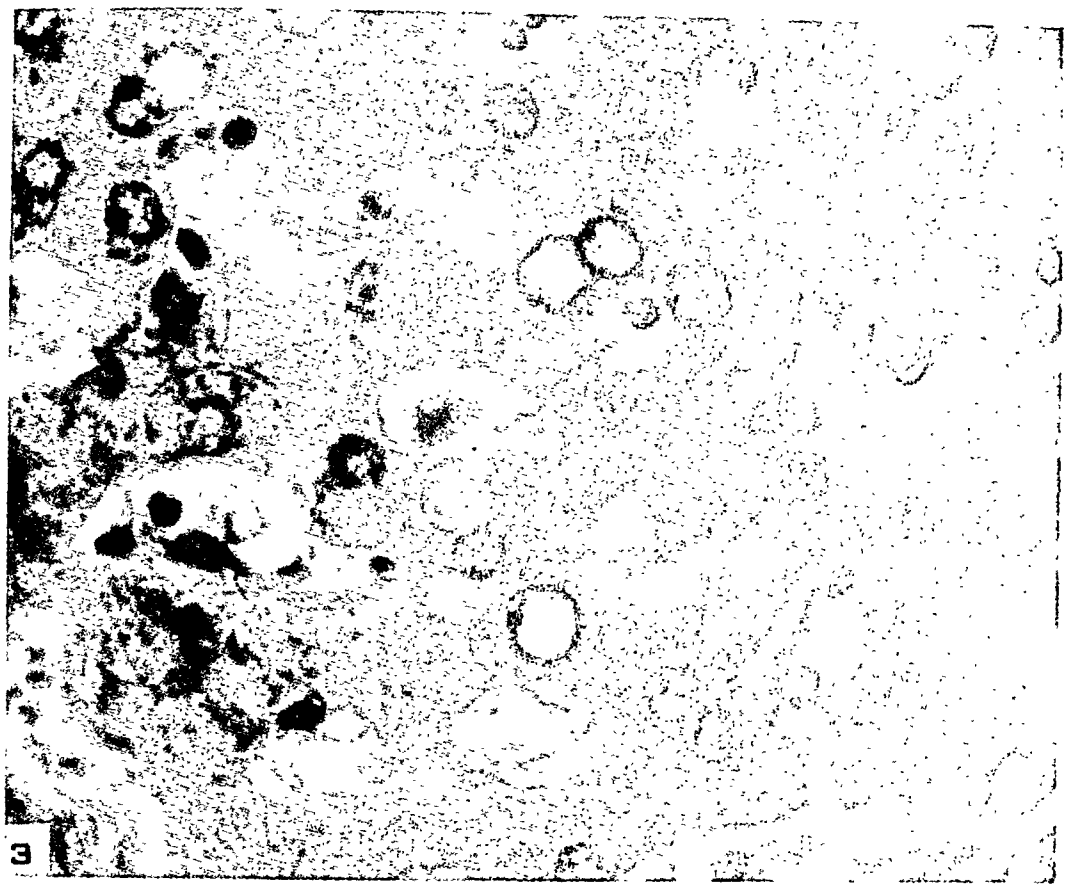
Glycogen Infiltration of Liver Cell Nuclei

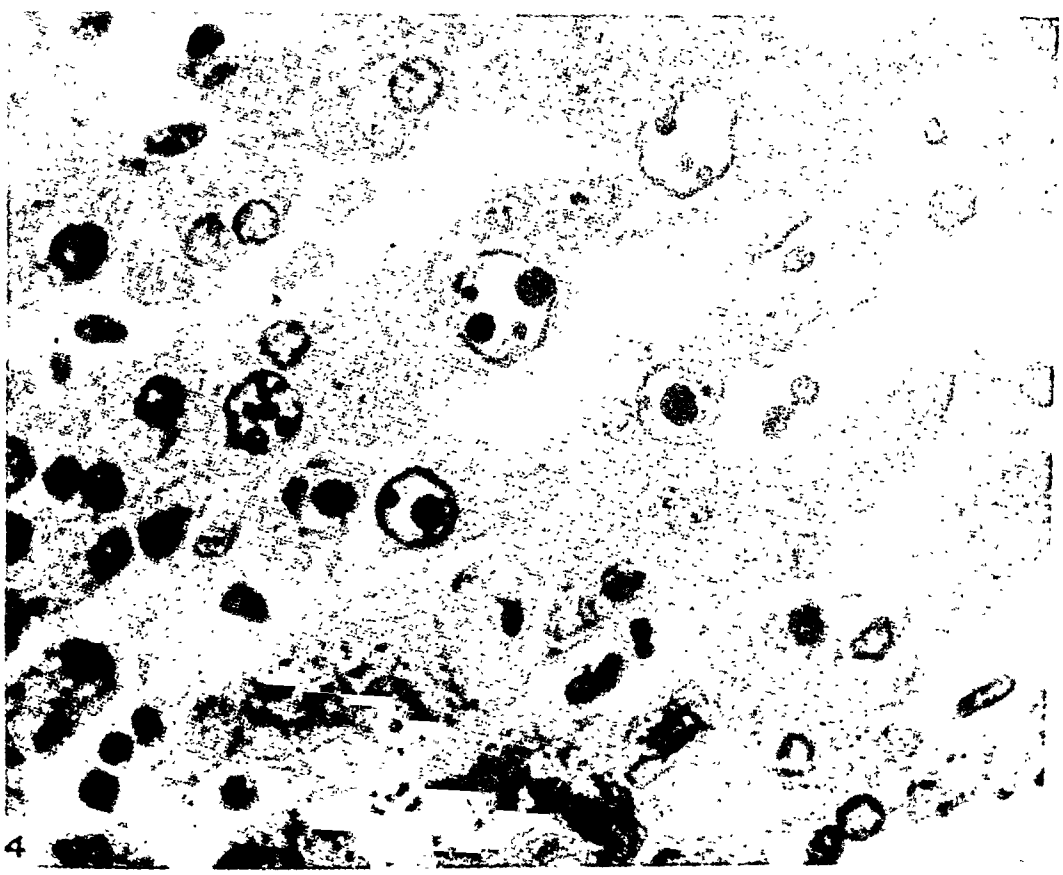
DESCRIPTION OF PLATES

PLATE 101

FIG. 1. Paraffin section of liver stained with haematoxylin and eosin, showing a patch of liver cells above in which the pale, fluffy cytoplasm is indicative of a rich glycogen content. These contrast with glycogen-poor cells below in which the cytoplasm has the more usual uniformly granular appearance. × 565.

FIG. 2. Paraffin section of liver stained with haematoxylin and eosin, showing large numbers of vacuolated, empty-appearing nuclei, particularly abundant near the periphery of the lobule. × 350.





Chipps and Duff

Glycogen Infiltration of Liver Cell Nuclei

PLATE 102

FIG. 3. Paraffin section of liver stained with haematoxylin and eosin, showing the characteristic vacuolated liver-cell nuclei. Chromatin and nucleoli occupy an extreme peripheral position. × 855.

FIG. 4. Celloidin-treated, paraffin section of liver stained by Best's carmine method, showing glycogen infiltration of six of the liver-cell nuclei. The crimson-stained glycogen is represented in the photomicrograph by the black intranuclear masses. Apart from the presence of these droplets of glycogen, the appearance of these nuclei is identical with that of the vacuolated nuclei shown in Figure 3. × 855.

EFFECTS OF YEAST AND FOOD INTAKE ON EXPERIMENTAL CARBON TETRACHLORIDE CIRRHOSIS OF THE LIVER IN THE RAT *

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Clinical studies on cirrhosis of the human liver have indicated that beneficial effects are produced by feeding a highly nutritious diet supplemented with vitamin B concentrates.[1,2] Nakahara, Mori and Fujiwara [3] have stated that the addition of baker's yeast to the diet protects the rat against the development of hepatic cirrhosis and hepatomas produced by p-dimethyl-amino-azo-benzol. This has been confirmed with brewer's yeast by Sugiura and Rhoads.[4] Von Glahn and Flinn [5] have reported that feeding brewer's yeast partially protects rabbits against cirrhosis produced by feeding lead arsenate. That liver damage may be produced by a diet deficient in factors contained in yeast has been indicated by György and Goldblatt [6] for the rat and by Rich and Hamilton [7] for the rabbit. Recently other reports suggest that other food components may be involved in the production of experimental cirrhosis.[8]

The present report describes the effects of the administration of brewer's yeast † on CCl_4 cirrhosis in the rat. Both developmental and recovery stages of the disease were studied.

It is well known that yeast stimulates the appetite (Cowgill,[9] Drill and Sherwood [10]). In the present studies, therefore, the effect of increased food consumption due to the appetite-stimulating action of yeast was controlled. This was done by feeding the same amount of food to the animals while varying the quantities of yeast in the diet. In this manner the essential variable of the experiment was the quantity of yeast consumed.

PART I. EFFECT OF FEEDING BREWER'S YEAST ON THE DEVELOPMENT OF CIRRHOSIS OF THE LIVER INDUCED BY CCl_4 IN THE RAT

Two sets of experiments were performed. The first, series I, was a comparison of the effects of 1.5 per cent to 1.0 per cent with those of 8 per cent to 10 per cent brewer's yeast supplements. The former concentration was found to be adequate for good growth in normal animals. The second set of experiments, series II, was a comparison of the effects

* Received for publication, November 3, 1941.

† Harris' medicinal brewer's yeast powder, Tuckahoe, N.Y.

central areas. Fibrosis in the liver lobule also began in the center and later extended to other areas. Therefore, the estimation of the degree of fibrosis, liver-cell vacuolization, necrosis and mitosis was made by the following methods: The central, portal and intercommunicating areas of the liver were individually graded with respect to fibrosis. The number of these anatomic areas was counted and the proportion of those which were fibrosed was determined. These were graded 1 to 4 for each anatomic area. Grade 1 signified that about 25 per cent of the specific areas was involved; grade 2, 50 per cent involvement, and grade 4, that all the specific areas were involved. The final value for fibrosis was the arithmetic sum of the grades for each anatomic area. Since three characteristics were graded; namely, central, portal and intercommunicating fibrosis, the maximum figure for a liver was 12.

The number of mitotic figures and necrotic cells per low power field was counted for three to ten fields. Vacuolization of liver cells was graded 1 to 4. Grade 1 indicated involvement of 25 per cent of the cells and 4 indicated involvement of every cell in the section. All slides were examined independently by two observers. Using the above standards, close agreement was observed. In this manner a reasonably quantitative evaluation of the lesions was made.

Results

Growth of Animals. Text-Figure 1 shows the average growth of animals of each of the four groups. No significant differences are seen between the growth curves of the two uninjected normal groups (A and B). The growth of these animals was about 1.5 gm. per day. Therefore, the ingestion of large excesses of brewer's yeast *per se* does not significantly affect the growth of normal rats.

The CCl_4 treatment depressed the growth rate of the rats in both groups C and D. However, group C, fed 8 to 10 per cent yeast, grew about twice as much as group D which was fed 1.5 to 1.0 per cent yeast. It may be concluded, therefore, that the ingestion of large amounts of brewer's yeast (8 to 10 per cent) afforded some protection against the growth-inhibiting action of CCl_4.

Histopathology of the Liver. Cirrhosis occurred in 95 per cent of the injected rats. However, in both CCl_4-injected groups there was marked variation in the liver lesions. Because of this variation the arithmetic means of the grades of the lesions were calculated. These means, recorded in Table I, are not significantly different. Figure 1 is a photomicrograph of a representative lesion for the livers showing moderate cirrhosis. The livers of the rats which did not receive CCl_4 appeared normal. In summary, it appears that in spite of its beneficial

of 0.75 per cent to 0.2 per cent with those of 10 per cent brewer's yeast supplements. The lower concentration was inadequate for good growth in normal animals.

SERIES I

Methods

Male rats of Sherman stock, 10 weeks old, were employed. They were injected subcutaneously with a 50 per cent solution of CCl_4 in mineral oil, twice weekly, in a dosage of 0.05 cc. per 100 gm. of body weight. The basal diet employed was: sucrose, 67 per cent; casein, 20 per cent;* butter, 8 per cent; Hawk-Oser [11] salt mixture, 4 per cent. One group of animals received this basal diet to which was added brewer's yeast in amounts of 1.5 per cent decreasing to 1.0 per cent. Another group of animals received the same basal diet except that the casein was 18 per cent; the sucrose, 62 to 60 per cent, and the brewer's yeast, 8.0 to 10 per cent. The decrease in the casein and sucrose concentration corrected for the additional protein and caloric values of the large yeast supplements.[12] To determine the influence of the diets on growth, two groups of rats received the same diets without being treated with CCl_4. This also offered an opportunity to study the effects of the administration of CCl_4 on the growth of rats.

The animals were grouped as follows:

Group A, 5 rats. No CCl_4. Basal diet and brewer's yeast, 1.5 per cent decreasing to 1 per cent.

Group B, 5 rats. No CCl_4. Basal diet and brewer's yeast, 8 per cent increasing to 10 per cent.

Group C, 20 rats. CCl_4. Basal diet and brewer's yeast, 8 per cent increasing to 10 per cent.

Group D, 20 rats. CCl_4. Basal diet and brewer's yeast, 1.5 per cent decreasing to 1 per cent.

The caloric intake was essentially the same for all rats and the daily food consumption per rat averaged 14 gm. This was achieved by limiting the food consumption of all animals to that of the animals in group D. The latter were fed *ad libitum*. Each animal was caged separately, fed and watered daily and weighed twice weekly.

After 11 weeks (or 22 injections of CCl_4) all rats were sacrificed after stunning and histological studies of the livers were made.

Tissues were fixed in Zenker's solution for hematoxylin and eosin and azan-carmine stains. Following CCl_4 administration, the earliest tissue changes, such as fatty degeneration and necrosis, were seen in the

* Grade 20, edible; Casein Co. of America.

SERIES II

In the next set of experiments, a comparison was made of the effects of feeding amounts of yeast (0.75 to 0.2 per cent) inadequate for good growth of normal rats with the effects of feeding amounts of yeast (10 per cent) in excess of that required for good growth. As in the foregoing experiments, all animals were fed the same amounts of food.

TABLE II

Series I. Effect of CCl_4 Injections and Yeast Intake on Liver Fat Content. High Yeast Feeding Diminishes the Elevated Liver Fat of CCl_4 Cirrhosis*

Group	Yeast (per cent)	Animals	Fat (per cent)		Differences in fat content		
			Mean	Standard deviation	Compared groups	Difference of means†	P‡
A, normal	1.5–1.0	5	4.0	±0.9	A and B	1.4	1/12
B, normal	8–10	5	2.6	±0.9	C and D	*1.5*	1/88
C, CCl_4	8–10	20	6.7	±3.2	B and C	*4.1*	<1/5000
D, CCl_4	1.5–1.0	20	8.2	±3.0	A and D	*4.2*	<1/5000

* Ether soluble substances.
† Significant differences are italicized.
‡ P value of less than 1/20 is statistically significant.[17]

Methods

Male rats, 10 weeks old, from the same stock as those of the previous experiment, were employed. CCl_4 was administered in the manner previously described. The basal diet for the rats receiving 0.75 to 0.2 per cent yeast was: sucrose, 65.25 per cent; casein, 22 per cent; butter, 8 per cent, and Hawk-Oser [11] salt mixture, 4 per cent. The 10 per cent yeast diet contained 60 per cent sucrose and 18 per cent casein to correct for the caloric and protein differences due to the high yeast supplements.

The effects of these diets on the growth of similar groups of rats not receiving CCl_4 also was determined.

The animals were grouped as follows:

Group A′, 10 rats. No CCl_4. Basal diet and brewer's yeast, 0.75 per cent decreasing to 0.2 per cent.

Group B′, 10 rats. No CCl_4. Basal diet and brewer's yeast, 10 per cent.

Group C′, 19 rats. CCl_4. Basal diet and brewer's yeast, 10 per cent.

Group D′, 20 rats. CCl_4. Basal diet and brewer's yeast, 0.75 per cent decreasing to 0.2 per cent.

effect on the growth of CCl_4-treated rats, high yeast ingestion did not modify the severity of the liver lesion.

Fat Content of the Liver. Fat determinations on the livers were made according to the method employed by Earle and Victor.[13] From Table II it is seen that the injection of CCl_4 was associated with an

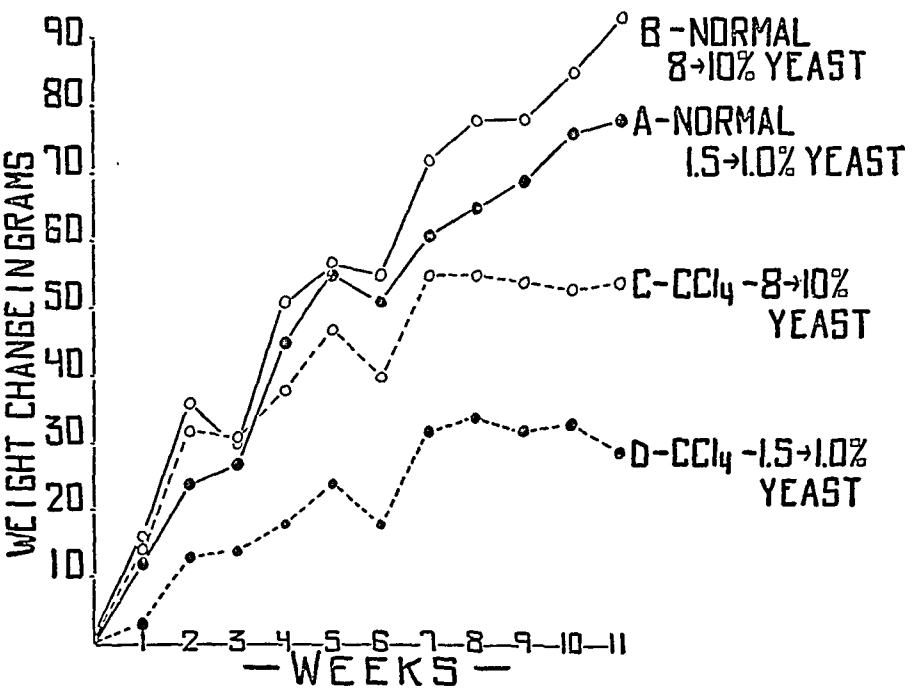
TABLE I

Liver Lesions of CCl_4-Treated Rats

Group	Dietary yeast (per cent)	Fibrosis, grade* 0–12	Vacuolization, grade* 0–4	Necrosis, no. of cells per low power field	Mitosis, no. of nuclei per low power field
Series I, C	8 –10	5.4	1.8	1.5	0.7
Series I, D	1.5 – 1.0	5.9	2.3	0.6	0.5
Series II, C′	10	7.0	1.8	†	1.1
Series II, D′	0.75– 0.2	7.8	1.8	†	1.2
Series III, E	1.77– 0.5	3.2	0.5	0	0
Series III, F	4.9 – 7.6	2.4	0.1	0	0
Series III, G	4.9 – 7.6	2.8	0.1	0	0

* The method used to estimate the grade of fibrosis and vacuolization is described in the text (see series I).

† Too many to count.



TEXT-FIGURE 1. Influence of CCl_4 and brewer's yeast on the growth of rats on isocaloric diets. Comparison of the effects of 1.5 to 1.0 per cent and of 8 to 10 per cent yeast. CCl_4 inhibited growth. Eight to 10 per cent yeast partially protected against CCl_4 inhibition of growth.

increased fat content of the liver. This confirms the findings of Winter.[14,15] However, the feeding of high yeast supplements (8 to 10 per cent) lessened to a limited, though significant degree, the lipoidosis of the liver resulting from CCl_4 administration.[16]

to 0.2 per cent (group D'). Figure 2 shows a characteristic lesion in these livers. Again, as in series I, in spite of its beneficial effect on the growth of CCl_4-treated rats, high yeast feeding did not modify the severity of the liver lesion.

TABLE III

Incidence and Degree of CCl_4 Cirrhosis at Two Levels of Food Intake as Indicated by Fibrosis

Degree of fibrosis*	Incidence per cent	
	Daily food consumption	
	Series I 14 gm. (40 rats)	Series II 11-8 gm. (39 rats)
None	5	5
Mild	25	15
Moderate	45	18
Severe	25	62

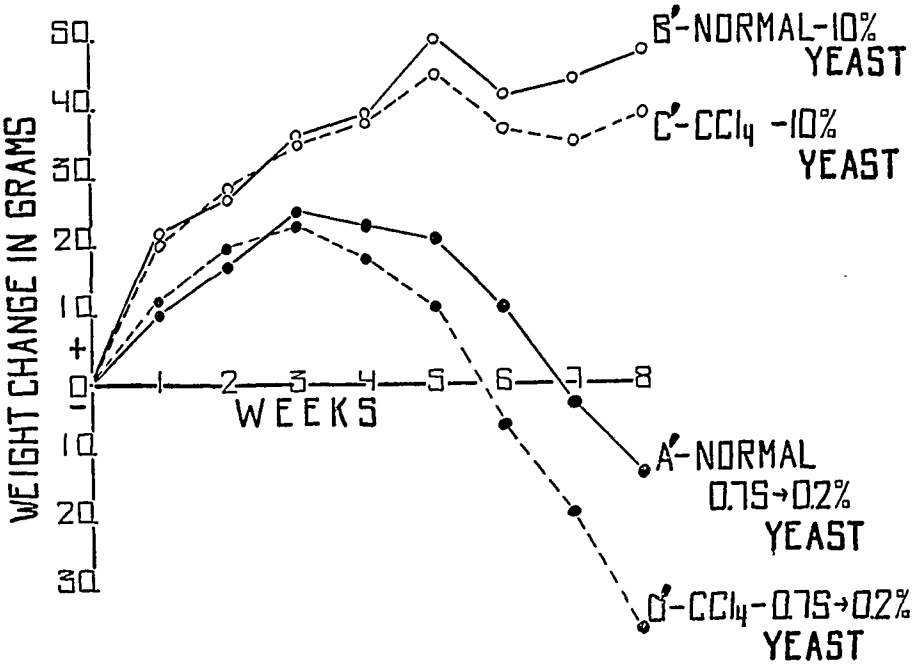
* See below for the method of grading the degree of fibrosis.

Effect of Differences in Food Consumption on CCl_4 Cirrhosis

The two series of experiments just described show that the feeding of yeast *per se* has no effect on the incidence of CCl_4 cirrhosis. Yet the average degree of fibrosis in the two series was different. Table III summarizes the incidence and severity of fibrosis in series I and II. The incidence is expressed in percentages, and the severity of fibrosis as none (0), mild (1 to 3), moderate (4 to 7), or severe (8 to 12).

Figure 1 is characteristic of the moderate hepatic fibrosis encountered in 45 per cent of the animals of series I and 18 per cent of those in series II. Figure 2 represents the severe cirrhosis found in 25 per cent of the animals in series I and 62 per cent of those in series II. The data show that the incidence of severe fibrosis was greater in series II: 62 per cent in contrast to 25 per cent in series I. These differences are greater than they appear to be, since the more severe lesions of series II occurred in animals treated with CCl_4 for a shorter time, namely 8 instead of 11 weeks, and the severity of hepatic cirrhosis is known to increase during CCl_4 treatment. The experimental conditions for these two series were the same except for the food intake. The food intake for series I was 14 gm. per day for each rat throughout the experiment; for series II it averaged 11 gm. for the first 4 weeks and 8 gm. for the last 4 weeks. At the lower level of food intake, CCl_4 produced more severe cirrhosis of the liver. It appears, therefore, that the severity of CCl_4 cirrhosis depends in part on the quantity of food consumed and not on the yeast intake.

The caloric intake of all rats was similar. This was achieved by limiting the food consumption of all animals to that of the animals in group D′. These latter animals were fed *ad libitum*. The daily food intake per rat was 11 gm. during the first 4 weeks and 8 gm. during the last 4 weeks. The experiment was discontinued at the end of 8 weeks (16



TEXT-FIGURE 2. Influence of CCl_4 and brewer's yeast on the growth of rats on isocaloric diets. Comparison of the effects of 0.75 to 0.2 per cent and 10 per cent yeast. CCl_4 inhibited growth. At this level of food consumption, 10 per cent yeast protected against CCl_4 inhibition of growth.

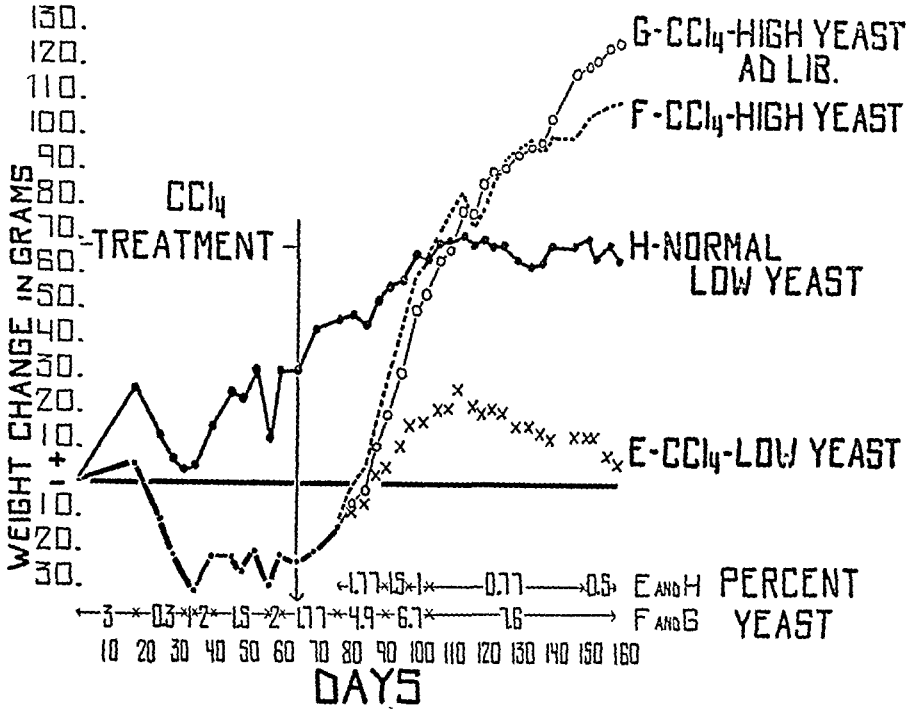
injections) because of the cachectic state of the rats in group D′, fed inadequate yeast. Morphological changes in the livers were evaluated as previously described.

Results

Growth of Animals. In Text-Figure 2 is shown the average growth of animals of each of the four groups. That the yeast supplements of 0.75 per cent decreasing to 0.2 per cent were inadequate for growth is shown by the poor growth of the uninjected rats in group A′. Again the growth-inhibiting effect of CCl_4 was manifest. On 10 per cent yeast supplement, both the CCl_4-treated and the uninjected rats grew better than those fed inadequate yeast, in spite of the fact that the caloric intake was the same in all these groups.

Histopathology of the Liver. The mean grades of the lesions are recorded in Table I. No significant differences were found between the lesions of the rats receiving 10 per cent yeast supplements (group C′) and those receiving 0.75 per cent yeast supplements, decreasing

25 per cent higher than that of the other groups. After 3 months of this regime all rats were sacrificed and their livers studied in the manner previously described.



TEXT-FIGURE 3. Influence of "low" and high brewer's yeast diets on growth after cessation of CCl_4 treatment. During the period of CCl_4 treatment growth was inhibited. After CCl_4 treatment was stopped, high yeast feeding accelerated growth much more than "low" yeast, in spite of isocaloric feeding.

Results

Growth of Animals. Text-Figure 3 shows the average growth of the animals described. During the 8 weeks of treatment the CCl_4-injected rats (lower line) grew less than the uninjected rats (upper line). After CCl_4 injections were discontinued all rats showed a sharp increase in the rate of growth. Those eating large amounts of yeast made a more rapid gain in weight than rats fed low yeast supplements even though the caloric intakes (excepting group G) were the same. This latter group, which was fed *ad libitum,* ate 25 per cent more food than the others, and attained a greater weight.

Histopathology of the Liver. The histological appearance of the livers is recorded in Table I. These data were derived in the manner already described.

The livers of these animals (groups E, F and G) showed far less fibrosis (Fig. 3), vacuolization and necrosis than was seen in the livers of animals sacrificed immediately after a course of CCl_4 treatment (Fig. 2). The liver scarring had almost entirely disappeared. Only

Part II. Effect of Feeding Brewer's Yeast on the Recovery from Hepatic Cirrhosis Induced by CCl_4 in the Rat

Methods

Fifty-one male rats, 10 weeks old, of the same stock employed in Part I, were injected subcutaneously with CCl_4 in the manner previously described. The injections were made twice weekly for 8 weeks. Eight additional rats, living under the same conditions, were fed the same diet but received no CCl_4.

During the period of CCl_4 administration, all rats received the following diet: sucrose, 67 per cent; casein, 18 per cent, and Hawk-Oser[11] salt mixture, 4 per cent, containing 0.24 per cent nicotinic acid. This diet was supplemented with small amounts of brewer's yeast (Text-Fig. 3). The animals were fed *ad libitum* and the food consumption was not measured during this 8-week period. The rats were kept two in a cage and fed and watered daily.

During this 8-week period, 7 of the animals receiving CCl_4 died. Six had severe cirrhosis of the liver and 1 could not be examined because it had been eaten by its cage mate. At the end of this period 8 CCl_4-treated rats were sacrificed along with 2 untreated rats. All 8 injected rats had severe cirrhosis. The 2 uninjected rats had normal livers. From the findings of severe cirrhosis in the 8 sacrificed rats just described, it was assumed that the remaining CCl_4-treated animals also had cirrhosis of the liver. The surviving 36 CCl_4-treated rats were then studied for the influence of dietary brewer's yeast on the possible recovery from the CCl_4 cirrhosis. Below is listed the grouping of the CCl_4-treated and untreated animals (series III).

Group E, 11 rats, "cirrhotic." Basal diet* and brewer's yeast, 1.77 per cent decreasing to 0.5 per cent; fed *ad libitum.*

Group F, 15 rats, "cirrhotic." Basal diet and brewer's yeast, 4.9 per cent increasing to 7.6 per cent. Food intake limited to that of group E.

Group G, 10 rats, "cirrhotic." Basal diet and brewer's yeast, 4.9 per cent increasing to 7.6 per cent; fed *ad libitum.*

Group H, 6 rats, normal. Same diet as group E; fed *ad libitum.*

All rats were caged separately during this stage of the experiment and were fed and watered daily. Group F was fed isocalorically with group E. Group H, although fed *ad libitum,* also consumed the same amount of food as did group E. Thus groups E, F and H had the same food consumption. The food consumption of group G, fed *ad libitum,* was

* Same basal diet as described above.

Summary and Conclusions

1. The effect of different levels of brewer's yeast ingestion on the hepatic cirrhosis produced by CCl_4 injections was studied in isocalorically fed rats. The lesions were studied both during the developing and healing stages of the disease.

2. The development of and recovery from cirrhosis of the liver was essentially the same whether amounts of yeast fed were grossly inadequate, adequate or in excess of the requirements for normal growth.

3. The addition of large amounts of brewer's yeast to the basal diet overcame in part a growth-inhibiting effect caused by CCl_4 injections.

4. The addition of large amounts of brewer's yeast to the basal diet decreased the lipoidosis of the liver caused by CCl_4 injections.

5. In contrast to the yeast intake, the food consumption modified the severity of the cirrhosis. Those fed 8 to 11 gm. of food daily had more severe liver lesions than those fed 14 gm. daily, regardless of the amount of yeast supplement.

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delicate strands of connective tissue remained, forming intercommunications between the liver lobules, which were regular in size and shape. The liver cells had returned to their normal size. No increase in bile ducts was present. In other words, there was considerable recovery from the CCl_4 cirrhosis 3 months after stopping the CCl_4 injections. However, in spite of the marked differences in growth, no significant morphological differences could be observed between the amount of healing of the cirrhotic livers of rats fed large (4.9 to 7.6 per cent) or small (1.77 to 0.5 per cent) amounts of brewer's yeast supplement. Under the conditions of these experiments, the 25 per cent greater food consumption of group G did not influence the degree of recovery of the cirrhotic liver. Again, as in the other series, the beneficial effect of high yeast feeding on growth was not associated with any modification of the liver lesion.

Discussion

Administration of CCl_4 depresses the growth of rats. Under conditions of isocaloric food intake, high yeast feeding improves the growth and general appearance of CCl_4-treated rats but does not influence that of normal rats. This implies that CCl_4 treatment increases the yeast requirements for growth.

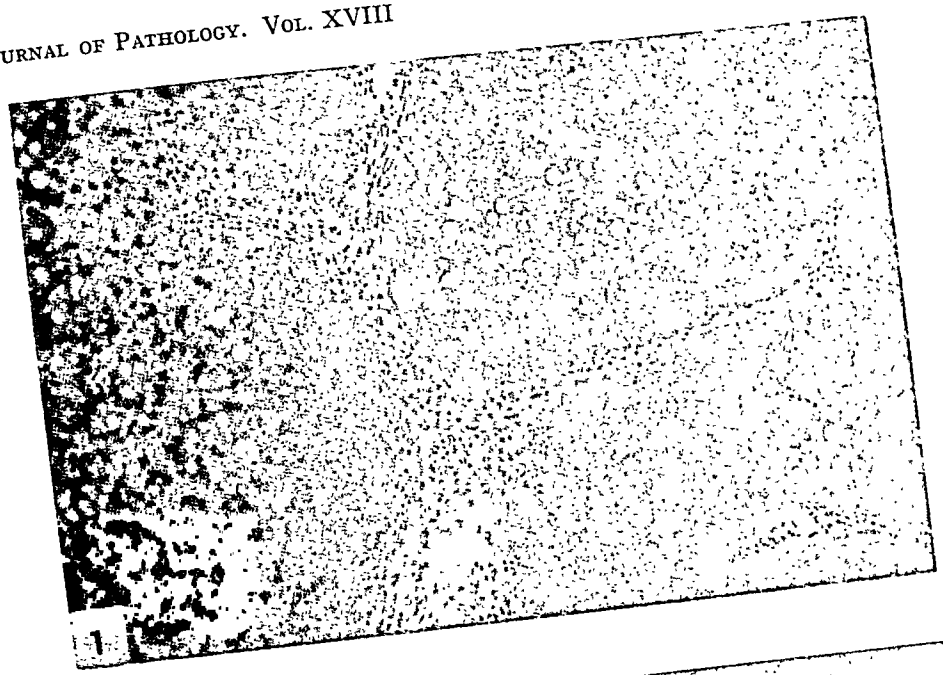
In these experiments there was no correlation between the growth of animals, as influenced by yeast, and the extent of the hepatic CCl_4 lesion. Therefore, growth cannot be used as an index of the modification of this disease process. Whether or not this conclusion is applicable to other disease processes seems worthy of consideration.

Brewer's yeast *per se* had no effect on the developmental or recovery stages of CCl_4 cirrhosis. When the food consumption was diminished either by artificial restriction or by anorexia due to inadequate yeast intake, the liver lesions due to CCl_4 were more severe.

In the present studies the amount of CCl_4 injected was kept constant. It is possible that with the administration of smaller amounts of CCl_4 a specific effect upon the liver lesions could be exerted by yeast in addition to its influence on the food intake.

The finding of the lack of any specific effect of yeast on CCl_4 cirrhosis is different from the protective effect of yeast observed with other hepatotoxins.[3-5] This may be related to differences between the hepatotoxic agents. On the other hand, the reported protection may have been due in part to the appetite-stimulating effect of yeast [9,10] with resulting increase in the food intake. From the present studies it would seem essential to control the food intake in this type of investigation.

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Effects of Yeast on Hepatic Cirrhosis

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ADDENDUM

Since this paper was submitted, a number of papers have appeared which describe the experimental production of cirrhosis of the liver by means of deficient diets:

Blumberg, Harold, and McCollum, E. V. The prevention by choline of liver cirrhosis in rats on high fat, low protein diets. *Science*, 1941, **93**, 598–599.
Daft, F. S.; Sebrell, W. H., and Lillie, R. D. Production and apparent prevention of a dietary liver cirrhosis in rats. *Proc. Soc. Exper. Biol. & Med.*, 1941, **48**, 228–229.
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DESCRIPTION OF PLATE

PLATE 103

FIG. 1. Moderate CCl_4 cirrhosis. Fibrosis about the central areas with some extension of the scars to adjacent central areas. Hypertrophy and vacuolization of the liver cells. Hematoxylin and eosin stain. × 100.

FIG. 2. Severe CCl_4 cirrhosis. Intercommunicating bands of dense scar tissue are seen, with newly formed bile ducts and hypertrophy and vacuolization of the liver cells. Hematoxylin and eosin stain. × 100.

FIG. 3. Healing CCl_4 cirrhosis. Delicate connective tissue strands outlining the liver lobules by communication between central and portal areas. Liver cells of normal size. Hematoxylin and eosin stain. × 100.

PRIMARY CARCINOMA OF THE LIVER *

CHOLANGIOMA IN HEPATOLITHIASIS

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From the standpoint of its pathogenesis, primary carcinoma of the liver, including both hepatoma and cholangioma, has been linked to a number of preëxisting hepatic lesions. Among these are cirrhosis (Laënnec's, pigment, toxic, obstructive biliary), parasitic infestation (clonorchiosis, distomiasis, schistosomiasis, echinococcosis), syphilis, congenital rests, chronic venous congestion, cholangitis and hepatolithiasis.

In the following two cases we report the rare occurrence of cholangioma, discovered as an incidental finding at autopsy, with hepatolithiasis associated with cholangitis and cholestasis.

REPORTS OF CASES

CASE 1

M. O., a white married housewife, 61 years of age, was admitted to the Buffalo General Hospital (service of Dr. Clayton W. Greene and Dr. Benjamin Jacobson) on September 23, 1938, because of stupor of 4 days' duration. She died in 12 hours.

In December, 1934, the patient fell down two steps and injured her back. At this time diabetes mellitus was diagnosed. In March, 1936, the patient was admitted to the hospital for pain in the knees. Upon discharge, the diagnoses were rheumatoid arthritis; diabetes mellitus; calcification of the arteries of the feet; compression of the twelfth dorsal vertebra and lipping of the eleventh, twelfth dorsal and first lumbar vertebrae; incisional hernia. There was no history of jaundice. On September 19, 1938, the patient became stuporous.

Examination. The temperature was 101.2° F. per rectum. The patient was stuporous but able to carry out orders. The eyeballs were soft. The abdomen showed an incisional hernia. Urinalysis gave a positive Benedict reaction. Before death the temperature reached 106° F. per rectum; cyanosis became prominent.

Autopsy Findings

The autopsy was performed 2 hours after death. The pathologic diagnoses were: early diffuse and patchy pneumonia of the right middle and lower lobes; inflammatory hyperemia of the right upper lobe; hepatolithiasis with dilatation, in places localized, of intrahepatic bile ducts containing thick, brown, sandy, mucopurulent bile and showing in some ducts papillary formations of the lining; a small papillary and solid bile duct carcinoma (cholangioma) of right lobe of liver; chronic cholangitis of extrahepatic bile ducts with choledocholithiasis

* Received for publication, September 4, 1941.

gross serial sections this papillary growth was found to fill completely the lumen of the duct, where it was less dilated proximal to the stone, and apparently to invade the wall. Here, surrounding the duct, especially anteriorly and medially, were several solid, white-yellow nodules, separated by gray-yellow septa. These nodules measured, with the duct included, 2.5 by 2.5 cm.

The capsule of the liver above the gallbladder was fibrosed. The gallbladder was dilated, measuring 13 by 10 cm. The serosa was fairly smooth. The wall measured 1 mm. in thickness. The mucosa was smooth, atrophic, showed no bile imbibition and its content was mucoid. The extrahepatic bile ducts were distinctly dilated: hepatic duct, 3 cm. in circumference; cystic duct, 1.7 cm., and common bile duct, 3 cm. The lining of the extrahepatic bile ducts was smooth. The walls were opaque and thickened. The bile was mucopurulent, brown and thick. The stones found in the extrahepatic bile ducts and gallbladder were small, averaging 0.9 cm. in diameter and were of the faceted, combination type.

The pancreatic duct opened above the common bile duct. In the head and body it was dilated to 1 cm. in circumference and contained brown gravel. The parenchyma of the pancreas was atrophic. The lymph nodes were free of metastases.

Microscopic Findings in Liver

The findings in the bile ducts were varied. In sections taken from the right lobe, the small bile ducts were lined by a single layer of high cuboidal epithelium. There was a distinct periductular fibrosis. Cellular infiltration in the portobiliary spaces was slight or minimal. The dilated ducts and cystic-like cavities from both lobes revealed atrophy or absence of epithelial lining. The walls were markedly thickened and fibrosed; they were only slightly inflamed.

The white papillary formations noted grossly in several dilated ducts and cavities distant from the tumor were of different types. In two ducts they were dependent upon a papillary and polypous inflammatory hypertrophy of the mucosa. Here glandular epithelium showed mitotic figures and large dark nuclei. In other ducts adenomatous hyperplasia of typical and also of atypical nature had occurred. In one, the basis for the papillary formation was papillomatous and adenomatous proliferation of the mucosa and wall. Here proliferations of the lining were rather cellular and contained little stroma. In a number of epithelial cells mitotic figures and atypical nuclei were found. Invasion of the wall was not demonstrated.

In contrast to the dilated ducts and cavities with atrophic or absent

and marked dilatation of the lumina; chronic cholecystitis with cholelithiasis; dilatation of pancreatic duct with atrophy of pancreatic tissue, fatty infiltration and interstitial fibrosis; hypertrophy of islets of Langerhans; postmortem blood sugar, 266 mg. per 100 cc., and CO_2-combining power, 16 mm. of mercury (diabetes mellitus); atherosclerosis; atrophy of the brain (950 gm.); myocardial fibrosis; compression of anterior surfaces of bodies of eleventh and twelfth dorsal vertebrae; leukoplakia of esophagus; healed laceration of cervix; fibrous pleurisy; incisional hernia; absence of appendix.

Gross Findings in Liver and Pancreatico-Biliary Tract

When the abdominal cavity was opened, the superior and anterior surfaces of the liver were found to be adherent to the inferior surface of the right hemidiaphragm by fibrous adhesions. Upon separation of these, the hepatic surfaces showed circumscribed, white-yellow, opaque elevations which measured up to 3.5 cm. in diameter. Palpation of these elevations elicited crepitus. The liver was reduced in size. The right lobe measured 17 by 15 by 8.5 cm.; it possessed an accessory cleft; the left lobe measured only 5 by 6 by 3 cm. On section, the cut surface revealed marked dilatation of the larger intrahepatic bile ducts with the formation of cavities along the course of the ducts. The white-yellow elevations, noted externally, corresponded to walls of cavities which reached the surface of the liver. In the left lobe such dilatation and cavitation was so marked that the parenchyma had almost disappeared from compression atrophy.

The dilated ducts and cavities measured up to 3.5 by 1 cm. Their walls were thickened. The content was composed of thick, brown, mucopurulent, sandy bile and stones. Smears revealed a variety of organisms, chiefly gram-negative bacilli and gram-positive cocci. For the most part, the stones were soft, brown and easily pulverized. They ranged in size from that of a pea to a pistachio nut. Multiple small faceted stones were also found in ducts. Chemical analysis of stones was positive for bile pigment and calcium. In some ducts and cavities stones fitted tightly. While in the main it appeared atrophic, the lining of several ducts and cavities in scattered locations demonstrated soft, low papillary formations without invasion of walls. The hepatic parenchyma was not icteric; the smaller portobiliary spaces were prominent.

On one gross section through the liver, there was discovered, practically by accident, in the anterosuperior medial portion of the right lobe (Fig. 1), a ductular cavity 1.8 by 1.8 cm. which presented, proximal to the site of lodgment of a large stone, a white, papillary growth into the lumen of greater extent than had been previously seen. On

power examination, in certain areas cords of carcinoma cells combined and merged with surrounding liver cells which showed large dark nuclei.

In the hepatic parenchyma the lobular markings were preserved. There were distinct regressive lesions in liver cords—hydropic and granular degeneration, necrosis of cells and dissociation of cords—distributed in both central and peripheral zones. Cells with large and double nuclei were seen. Surrounding dilated ducts and cavities, the hepatic parenchyma was compressed and atrophic. The walls of small arteries were thickened and hyalinized. In medium-sized arteries intimal sclerosis was present. In large vessels the internal elastic layer had become calcified in part. The lymph nodes showed chronic, nonspecific lymphadenitis but no metastases.

CASE 2

W. M., a white man, 62 years of age, was admitted to the Niagara Falls Memorial Hospital (service of Dr. George Stoll) on December 27, 1939, with chills and fever. He died January 15, 1940.

For 3 weeks the patient had suffered with epigastric pain. One day before admission he developed fever and chills. There were abdominal distention and vomiting. About 1 year previously the patient had had two operations on his biliary tract.

Examination. The skin was icteric. Râles were heard in the left lower chest. The heart was slightly enlarged. A systolic murmur was heard at the mitral area. The abdomen, which showed a scar in the right upper quadrant, was distended and tympanitic. The liver seemed to be enlarged. The left arm and leg were paralyzed.

Laboratory studies disclosed: hemoglobin, 55 (Newcomer); red blood cells, 2,850,000 per cmm.; white blood cells, 21,200 per cmm. with 88 per cent neutrophils, 10 per cent lymphocytes and 2 per cent transitionals; icteric index, 9. Urine: specific gravity, 1008; albumin, 1 plus; few red blood cells; many white blood cells. Blood culture was negative. The temperature ranged from 98.6° to 106° F. Terminally, cough and cyanosis appeared.

Autopsy Findings

The autopsy was performed 1 hour after death. The pathologic diagnoses were: status following hepaticoduodenostomy; abdominal scars; choledocholithiasis of common bile, cystic and hepatic ducts; chronic cholecystitis; hepatolithiasis, chronic cholangitis of hepatic and intrahepatic ducts; small cholangioma of right lobe of liver; purulent thrombophlebitis of portal and superior mesenteric veins; multiple pylephlebitic abscesses of liver; metastatic abscesses of left lung with fibrinous pleuritis; purulent bronchiolitis; diffuse and confluent bronchopneumonia of right lower lobe and slight serofibrinous pleuritis; pulmonary embolism and thrombosis of right upper lobe; chronic splenitis; leiomyoma of esophagus; solitary cyst of left kidney; chronic hemorrhagic cystitis; chronic prostatitis with atypical

lining, the ducts with epithelial and glandular proliferations were markedly inflamed. In the mucosa and wall there was distinct infiltration of neutrophils, mononuclear cells and plasma cells. Fibrosis of the wall was marked. Bile pigment was inspissated. Some of the small bile ducts in the vicinity of the carcinomatous site contained leukocytes, desquamated cells and granular débris. Rarely bile pigment was seen in the lumina. The lining membrane, where still preserved, was single-layered, with occasional hyperchromatic nuclei. The walls were infiltrated with cells of inflammatory origin. The portobiliary stroma was increased in amount and infiltrated with neutrophils, round cells and plasma cells.

On the medial margin of the carcinomatous site, three medium-sized and larger ducts, in addition to having exudate and desquamated cells in the lumen and active chronic inflammation in the wall, revealed marked papillomatous growth of the mucosa which practically filled the lumina. In these ducts there was a gradation in the character of the epithelial proliferation from a more or less typical form to atypical proliferation with large hyperchromatic nuclei, mitotic figures and early invasion.

The large duct in the central portion of the carcinomatous site showed, besides chronic nonspecific inflammation, marked papillomatous proliferation, and here in places the epithelial growth was altogether medullary and atypical. In the wall there was epithelial invasion of both solid and glandular types. The picture in this duct was that of a papillary, solid and glandular carcinoma (Fig. 2).

The nodules surrounding the duct just described were composed of solid carcinomatous alveoli and trabeculae. The cells were chiefly polyhedral with rather distinct outline and blue-staining cytoplasm. Nuclei were vesicular, oval, or round. Chromatin was arranged in small dots or nucleoli. Many mitotic figures and atypical nuclei were seen. No sinusoids ran between the cells. Alveoli and trabeculae within the nodules were separated by thin-walled vessels or by a slight amount of connective tissue. There were no multinucleated giant cells. The large nodules were separated by inflamed fibrous tissue. Throughout the solid alveoli and trabeculae cells tended to take cuboidal and columnar shapes and to form ductular and glandular structures suggestive of bile ducts. Lymphatic and perineural spaces were invaded by the tumor. In one area of solid carcinoma necrosis and hemorrhage had occurred. The blood vessels were dilated. In the stroma of the solid tumor macrophages contained brown pigment. On low power examination the carcinomatous site appeared well outlined. In places it was separated from the surrounding liver by connective tissue. On high

Microscopic Findings in Liver

There was a purulent thrombopylephlebitis. The huge abscesses with much fibrin were of pylephlebitic origin and some of these were multilocular. They were surrounded by thin walls of connective tissue infiltrated with round cells, neutrophils, plasma cells and eosinophils. The adjacent liver was compressed. The hepatic parenchyma in both lobes showed distinct obstructive-infective biliary cirrhosis. There was distinct icterus, particularly in the central zones, with bile in the canaliculi. The hepatic cells showed marked regressive changes. The lobules were small and the portobiliary tissue was increased in amount, with infiltration of neutrophils and round cells. Bile ducts were more numerous than normal and they were dilated. Their lumina contained bile pigment and concrements. The arteries showed intimal thickening. In the carcinomatous site, hepatic architecture no longer remained and findings in the bile ducts varied. Some ducts were dilated by impacted cholesterol pigment stones. The epithelium was atrophic or desquamated; it was also atypical. Other dilated ducts contained purulent exudate in the lumina, with red blood cells and desquamated epithelium. Bile pigment was also present. Ductular walls were fibrosed; they were infiltrated with neutrophils, plasma cells and round cells.

One large duct near the central portion of the carcinoma showed exudate and "stone" in the lumen and inflammation in the wall. For the most part, the lining of this duct was made up of a single layer of typical columnar cells. In one part of the lining, however, there was marked atypical proliferation with papillary formations and large hyperchromatic nuclei. In this area there was also invasion of the wall Similar atypical epithelial proliferation with invasion, associated with marked inflammation, was found in other large ducts (Fig. 3). The invading carcinoma represented various types of growth. For the main, it was a mature, papillary adenocarcinoma. In certain areas it was mucin-producing. In other places it was immature in character and contained giant cells. The surrounding liver tissue was invaded. Necrosis was moderate in amount. There was a moderate scirrhous reaction with inflammation and lipoid resorption. Perineural and lymphatic spaces and nerves were invaded. The tumor reached the capsule of the liver and the gallbladder bed. There was a marked chronic perihepatitis. Lymph nodes showed no metastases.

Comment

The question arises as to the possibility of a pathogenetic relationship between hepatolithiasis, with concomitant chronic biliary infection and cholestasis, and the primary carcinoma of the liver. In answering

epithelial proliferation; slight myocardial fibrosis; ascites; edema of legs. Smears and cultures from liver, portal vein and lungs revealed Friedländer's bacillus.

Gross Findings in Liver and Pancreatico-Biliary Tract

When the peritoneal cavity was opened, anatomic relations in the right upper quadrant could not easily be made out. There were marked fibrous adhesions involving the parietal wall behind the abdominal scar, the stomach, the duodenum and the liver, which was also adherent to the inferior surface of the right hemidiaphragm. The hepatoduodenal ligament was shortened. The pylorus was patent. The first portion of the duodenum was dilated. In its anterosuperior margin, 6.6 cm. from the pylorus, was an opening 0.8 cm. in diameter, from which escaped thick pus, bile and gravel. This duodenal opening communicated with the main hepatic duct which was 2.2 cm. in length and 1.8 cm. in circumference. The ductular wall was distinctly thickened. The lumen contained mucopurulent material, biliary gravel and stones.

The right and left hepatic ducts showed similar findings in the walls and lumina. They were dilated and apparently shortened. The common bile duct measured 8.2 cm. in length. It was dilated to 2.5 cm. in circumference and the wall was slightly thickened. The lumen contained multiple combination stones. The cystic duct, 1.5 cm. long, was contracted upon stones. The gallbladder was contracted, measuring 4 by 2.2 cm. Its wall was 0.2 cm. thick. There were no stones in it.

The right lobe of the liver measured 18 by 14 by 6 cm.; the left lobe, 16 by 7 by 7 cm. The right lobe appeared deformed. Shining through the thickened capsule of the liver were a number of soft yellow nodules. On section, these, as well as other nodules in the hepatic parenchyma, proved to be abscesses with walls of lipoid-containing granulations. The pus was non-fetid, thick and mucoid in character. The intrahepatic bile ducts were dilated. They contained pus, bile, gravel and combination stones of cholesterol-pigment type, and in some ducts, stones were lodged tightly. Stones were more numerous in the right lobe than in the left. On section through the right lobe of the liver there was discovered a dilated, thickened duct which contained stones but which was also filled with soft yellow, papillary growth. Similar growth was found in other ducts in this region. The entire area was about 2.5 cm. in diameter. The growth reached the posterior surface of the liver. It could be made out grossly that the papillary growth did not originate in the gallbladder or in extrahepatic bile ducts. No metastases were found in the nodes.

destruction or dilatation with flattening of the epithelium. Inasmuch as chemical analysis of bile from intrahepatic ducts was not made in our cases, we can offer no comment on specific changes in the composition of that bile which was not being evacuated normally from a liver with parenchymal damage, nor on the possible relation between these changes and the pathologic findings.

Summary

Primary bile duct carcinomas (cholangiomas) of the liver associated with hepatolithiasis, cholangitis and cholestasis were discovered as incidental findings at autopsy in a diabetic woman, 61 years old, and in a man, 62 years old, with history of biliary tract disease. The question of the pathogenetic relationship of hepatolithiasis with cholangitis and cholestasis to primary bile duct carcinoma of the liver is discussed.

Note: We are indebted to Dr. Kornel Terplan for his helpful criticism and to Dr. Clayton W. Greene and Dr. George Stoll for their permission to transcribe the clinical records.

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this question, our cases have particular significance because in each the carcinoma was only an incidental finding at autopsy. It gave no clinical symptoms. Its size was relatively small. Its origin and spread could still be fairly well inferred and traced. It produced no metastases.

Stones, along with the accompanying cholestasis and chronic inflammation, have been accepted as important factors in the development of carcinoma of the gallbladder.[1] Ewing[2] stated that in addition "to mechanical effect of calculi, presence of cholesterol and irritative and digestive action of the bile" play major pathogenetic rôles.

How far can the relation which exists between stones with cholestasis and chronic inflammation and carcinoma of the gallbladder be applied by analogy to carcinoma of the liver? In our cases intrahepatic bile ducts were dilated along their course into inflamed, fibrosed, cyst-like structures which contained stones and infected, thick, sandy bile. In case 1 a typical non-invasive, papillomatous and adenomatous proliferation occurred along with inflammation and lodgment of stones in ducts distant from the carcinomatous site. In both cases the carcinoma itself was histologically a cholangioma which could be directly related topographically and pathogenetically to an intrahepatic calculous cholangitis with marked papillary and glandular proliferation and pericholangitis (biliary cirrhosis).

Yamigawa[3] cited six cases (G. Kika) of carcinoma of the large intrahepatic bile ducts combined with cholangitis and pericholangitis exhibiting papillomatous and adenomatous hyperplasia of mucosa and wall. In one of these the inflammation and hyperplasia were caused by impaction of a biliary stone. Dahl[4] reported a case of cholangioma in paratyphoid cholangitis, cholecystitis, cholelithiasis and biliary cirrhosis, but in this instance we feel that it is difficult to evaluate pathogenetic relations. Rufanov[5] stated that cases of neoplasm of the ducts have been seen in hepatolithiasis, but he made no specific reference to a report of carcinoma of intrahepatic bile ducts associated with liver stones. According to Ewing,[2] carcinoma of intrahepatic bile ducts commonly yields a history of severe disturbance of evacuation of bile.

Certain experiments on the effects of different degrees of cholestasis on the lining of intrahepatic bile ducts are available. Partial ligation of bile ducts in dogs produces degeneration and desquamation of the epithelium in the large intrahepatic bile ducts.[6] Following complete aseptic ligation of the common bile duct or of an hepatic duct,[7,8] there develops high-grade papillary and adenomatous proliferation of the mucosa of medium-sized and large intrahepatic bile ducts with hyperchromatic nuclei, mitotic figures, inflammation and periductular fibrosis. The small ducts also proliferate. Subsequently the ducts undergo

destruction or dilatation with flattening of the epithelium. Inasmuch as chemical analysis of bile from intrahepatic ducts was not made in our cases, we can offer no comment on specific changes in the composition of that bile which was not being evacuated normally from a liver with parenchymal damage, nor on the possible relation between these changes and the pathologic findings.

SUMMARY

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this question, our cases have particular significance because in each the carcinoma was only an incidental finding at autopsy. It gave no clinical symptoms. Its size was relatively small. Its origin and spread could still be fairly well inferred and traced. It produced no metastases.

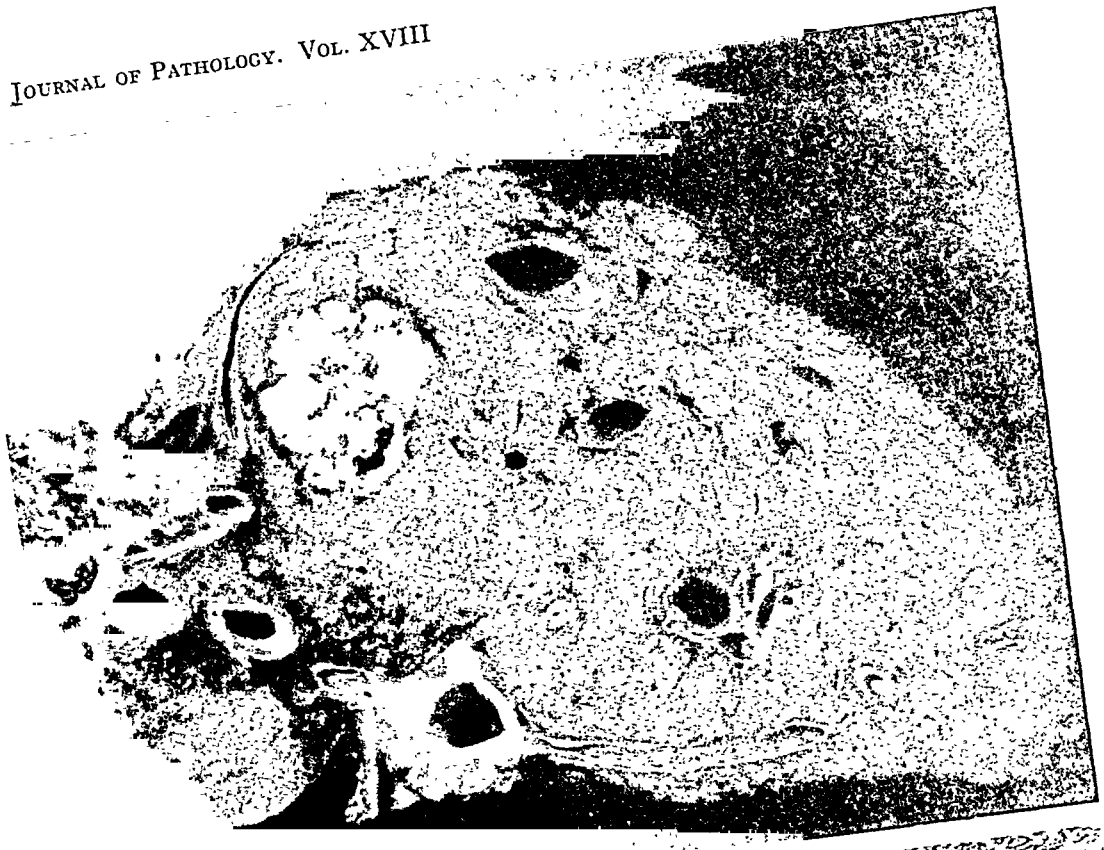
Stones, along with the accompanying cholestasis and chronic inflammation, have been accepted as important factors in the development of carcinoma of the gallbladder.[1] Ewing[2] stated that in addition "to mechanical effect of calculi, presence of cholesterol and irritative and digestive action of the bile" play major pathogenetic rôles.

How far can the relation which exists between stones with cholestasis and chronic inflammation and carcinoma of the gallbladder be applied by analogy to carcinoma of the liver? In our cases intrahepatic bile ducts were dilated along their course into inflamed, fibrosed, cyst-like structures which contained stones and infected, thick, sandy bile. In case 1 a typical non-invasive, papillomatous and adenomatous proliferation occurred along with inflammation and lodgment of stones in ducts distant from the carcinomatous site. In both cases the carcinoma itself was histologically a cholangioma which could be directly related topographically and pathogenetically to an intrahepatic calculous cholangitis with marked papillary and glandular proliferation and pericholangitis (biliary cirrhosis).

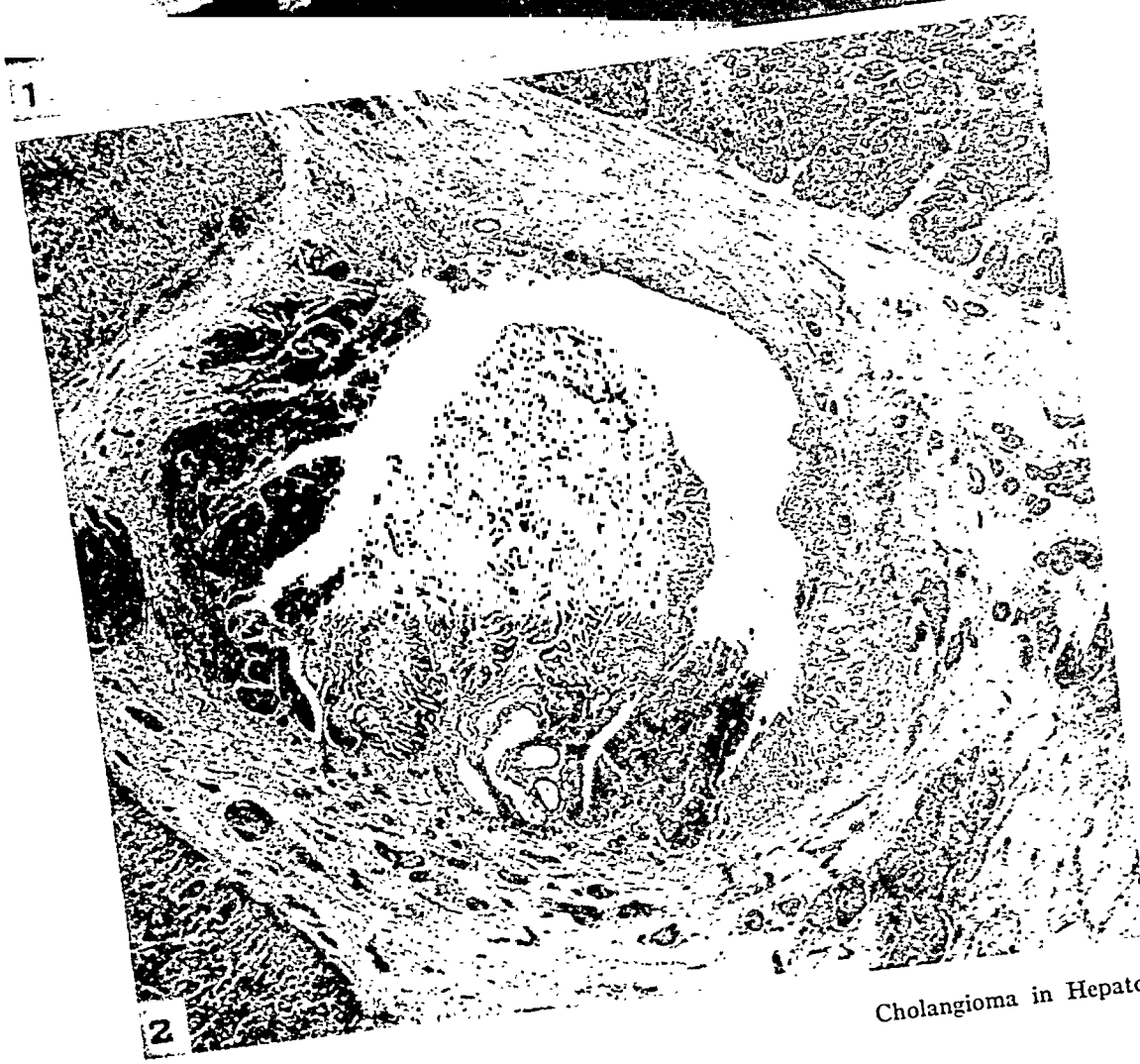
Yamigawa[3] cited six cases (G. Kika) of carcinoma of the large intrahepatic bile ducts combined with cholangitis and pericholangitis exhibiting papillomatous and adenomatous hyperplasia of mucosa and wall. In one of these the inflammation and hyperplasia were caused by impaction of a biliary stone. Dahl[4] reported a case of cholangioma in paratyphoid cholangitis, cholecystitis, cholelithiasis and biliary cirrhosis, but in this instance we feel that it is difficult to evaluate pathogenetic relations. Rufanov[5] stated that cases of neoplasm of the ducts have been seen in hepatolithiasis, but he made no specific reference to a report of carcinoma of intrahepatic bile ducts associated with liver stones. According to Ewing,[2] carcinoma of intrahepatic bile ducts commonly yields a history of severe disturbance of evacuation of bile.

Certain experiments on the effects of different degrees of cholestasis on the lining of intrahepatic bile ducts are available. Partial ligation of bile ducts in dogs produces degeneration and desquamation of the epithelium in the large intrahepatic bile ducts.[6] Following complete aseptic ligation of the common bile duct or of an hepatic duct,[7,8] there develops high-grade papillary and adenomatous proliferation of the mucosa of medium-sized and large intrahepatic bile ducts with hyperchromatic nuclei, mitotic figures, inflammation and periductular fibrosis. The small ducts also proliferate. Subsequently the ducts undergo

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Cholangioma in Hepatolithiasis

DESCRIPTION OF PLATES

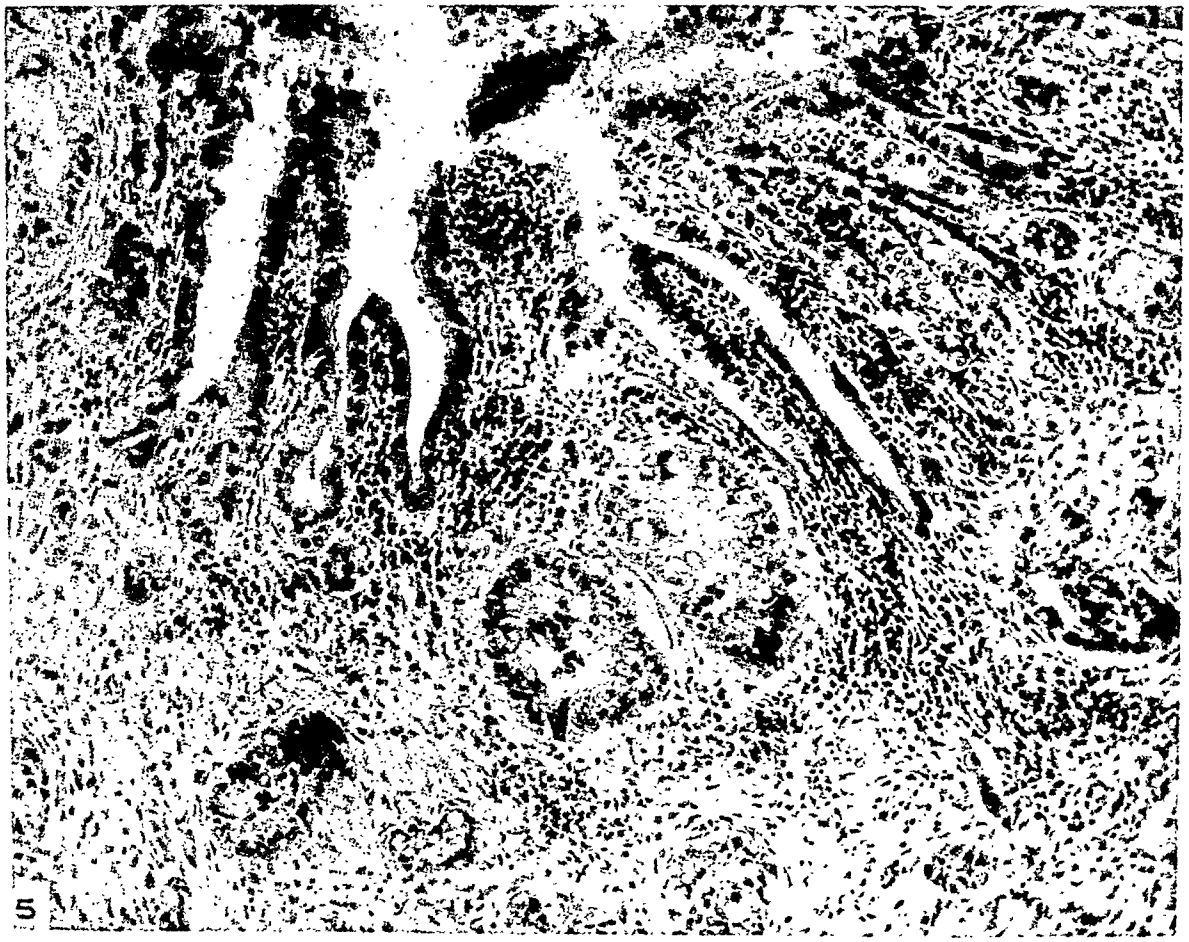
PLATE 104

FIG. 1. Case 1. Gross appearance of liver with small carcinoma of bile duct origin (cholangioma) and dilated bile ducts.

FIG. 2. Case 1. Low power view of cholangioma showing papillary, glandular and solid types. × 10.







Sanes and MacCallum Cholangioma in Hepatolithiasis

Plate 105

Fig. 3. Case 2. Low power view of bile duct showing papillary adenocarcinoma and inflammation. × 17.

Fig. 4. Case 2. Low power view of dilated bile duct with concrement. × 17.

Fig. 5. Case 2. High power view of bile duct showing invading adenocarcinoma. × 136.

BONE-MARROW CHANGES PRODUCED BY SPECIFIC ANTIBODIES *

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Animals injected repeatedly with heterologous tissue extracts are capable of producing antibodies against the original extracts. Such antibodies may be demonstrated in a number of ways. With this organ extract (antigen)-antibody technic, Masugi [1] produced progressively damaging lesions in the liver and kidneys. Antiplatelet antibodies, which reduced circulating platelets to zero, and purpura were demonstrated in animals by Ledingham.[2] Similarly acting antilymphocytic and antileukocytic serum has been made by Chew, Stephens and Lawrence.[3,4] Clinically and in experimental hemolytic anemia, Damesheck and Schwartz [5] showed that erythrocytes may be destroyed by anti-erythrocyte antibodies. Antibodies capable of producing abortion, apparently by their specific action on the placenta, were demonstrated in rabbits and in two human cases of spontaneous separation of the placenta by Cohen and Nedzel.[6] Precipitin methods have demonstrated antibodies against lung (Salfeld and Weichsel [7]). Pfeiffer,[8] as early as 1905, made the first recorded attempt to produce antitissue antibodies; he utilized spermatozoa. Burky,[9] using sensitivity methods, showed specific antibodies for lens and muscle. Bailey and Gardner,[10] by passive anaphylaxis, produced tissue specific antibodies for brain and medullated nerves. Although Schwentker and Rivers [11] were not altogether clear about the mechanism which produced extensive structural damage in the brains of rabbits by the injection of extracts of homologous brain, it seems likely that it was on an antigen-antibody basis.

The present study was essayed in order to determine the effect on the blood picture and bone marrow following single and multiple injections of heterologous bone-marrow antibodies.

MATERIAL AND METHOD OF PREPARATION

Macerated rabbit bone marrow was obtained by dissecting and crushing open heads of the long bones, flat bones, sternum, ribs and vertebral bodies and grinding with mortar and pestle. Slightly more than an equal quantity of distilled water was added and to this the same amount of cold ethyl ether. This was repeatedly shaken in the

* From a paper presented at the Twenty-Eighth Annual Meeting of The American Society for Experimental Pathology, Chicago, Illinois, April 19, 1941.

Received for publication, September 9, 1941.

those seen in the earlier group. The sharp reductions still occurred when antileukocyte serum was injected, with a relative lymphocytosis. However, when the injections were repeated over a period of time, the animals developed, in addition to, and following the leukopenia, a slight leukocytosis. Reductions in hemoglobin were common following a number of injections. One such animal, rabbit A in Table II, showed a profound anemia. Table II summarizes the peripheral blood studies of 2 animals which received repeated injections.

TABLE I

Total Leukocyte Counts

Animal	Pre-injection count (15 min.)	Amount injected (ml.)	Count after injection		
			5 min.	15 min.	24 hrs.
A	11,000	10	4,900	3,700	12,700
B	10,300	10	3,000	4,100	13,500
C	10,500	10	3,600	4,000	8,150
D	10,600	5	2,400	3,200	10,600
E	9,300	5	2,600	5,000	12,400
F	6,000	5		5,200	13,100
G	10,200	5	<100	4,300	11,000
H	8,000	5	4,200	1,500	8,600
I	7,600	5	3,500	4,300	11,300
J	9,100	8	<200	<200	9,100
K	8,800	8		5,000	10,200
L	10,800	8	3,900	4,600	13,000
Total averages for 12 animals given above	9,300	7	28,000	3,700	11,100

Typical Differential Count in Per Cent

	Polymorpho-nuclears	Basophils	Lymphocytes
5 min. after injection	5	1	92
15 min. after injection	0	0	97
24 min. after injection	14	1	85

Gross Bone-Marrow Findings

All of the bone marrow examined in the animals which received a single injection showed an active hyperemia and scattered, minute petechiae. In the animals which were injected repeatedly, hemorrhages were not prominent. Instead, there were many small yellowish gray flecks, apparently of necrosis. Also, there were larger gelatinous areas, and some areas of the bone, particularly near the epiphyses, were quite fibrotic. The spleens in all of the animals of this last group were greatly enlarged and on section showed a hyperplastic gray pulp.

cold for a period of 4 hours and the ether-fat fraction allowed to separate. The water extract was then drawn off and its protein rapidly precipitated by adding to 20 parts of the water extract, 80 parts of cold c. p. acetone. The precipitate was quickly centrifuged and the acetone decanted. The precipitate was then put into solution in a minimum of distilled water. This gave a cloudy gray-brown solution which was used for injections. Fresh solutions were made every 2 weeks. One to 2 ml. of this solution was injected intramuscularly into guinea pigs for 16 bi-weekly injections. The guinea pigs were bled out by cardiac puncture 10 days after their last injection. The pooled serum from their clotted blood was stored in an ice box and used as soon as possible.

The guinea pig antibone-marrow serum was injected in amounts ranging from 5 to 10 ml. into the lateral ear veins of rabbits whose blood picture previously had been proven to be normal and whose leukocyte count directly prior to injection was less than 11,000, with a normal differential count. Following the antibody injections, counts were done at 5, 10 and 15 minutes and 24 hours following injection. One group of animals received repeated antibody injections over a period of 2 months. On these, daily counts and differential studies were done. Biopsies were taken from time to time on both groups and autopsies finally performed. The usual routine autopsy examinations were done with particular reference to the microscopic sections of the upper femoral, pelvic and sternal bone marrow and spleen.

Results

Peripheral Blood Studies

All 12 animals which received a single injection of antibone-marrow serum showed an immediate leukopenia caused by reduction in circulating myeloid cells. The polymorphonuclear leukocytes may be driven to zero within 15 minutes following the injection. Thus there is a relative lymphocytosis which persists for 24 hours even though the total count has returned to near normal at that time. Other blood changes were not found before the animals were sacrificed for microscopic study at 72 hours. The results of the peripheral blood studies obtained in these experiments are summarized in Table I.

A second group of 12 animals were injected at intervals of 1 to 6 days over a period varying from 1 week to 2 months. The amounts injected varied from 0.5 to 5 ml. of antibone-marrow serum. In spite of considerable variation in time intervals of injection and in the amount injected, the peripheral blood studies were consistent with

were not immune to necrosis and, following a new antibody injection, were necrosed as extensively as was normal bone marrow. The fourth phase consisted of proliferation of fibrous connective tissue, scarring and cyst formation (Figs. 3 and 4). Early in the fourth phase young fibrous connective tissue growth was evident. This seemed to arise from the endosteal cells (Fig. 3). The heaviest connective tissue growth was found near the epiphyses. Cysts were found most often in the long bones. The substance in them stained a light pink.

The larger doses of antiserum produced more extensive changes than did smaller amounts.

Discussion

It seems clear that a single injection of specific antibody produces a direct change in the bone marrow which is reflected in the peripheral blood. This change, consisting of an indiscriminate necrosis of bone-marrow elements, is accentuated by repeated injections so that the end-picture is similar to that seen in osteitis fibrosa of humans. The peripheral blood picture shows a consistent primary leukopenia followed in later injections by a slight leukocytosis. It is not at present possible to state the mechanism of the bone-marrow necrosis. Its rapidity of occurrence is suggestive of direct damage by antibodies. However, Kay [12] has proposed a much more complicated mechanism in experimental antibody nephritis.

The usual occurrence of necrosis in the central portions of the bone marrow may be brought about because the perforating arteries supply the cortical bone marrow first and thus the central bone marrow is relatively anoxemic. Hyperplasia and regrowth adequately explain the leukocytosis encountered late in the protracted experiments. The appearance of hyperplastic areas along the periphery may mean that the bone marrow regenerates from this region. If this be true, we have another clue as to how bone marrow grows following damage in various conditions.

Summary

In summary, a method is given for obtaining antibodies against rabbit bone marrow. The injection of these specific antibodies causes an immediate reduction in circulating cells of bone marrow origin. The antibody acts not only on the circulating leukocytes but also directly on the bone marrow, producing in it hemorrhage, areas of acute necrosis, hyperplasia of the remaining bone marrow and finally extensive fibrosis and cyst formation. There is similarity of the experimental fibrotic lesions to those found in humans with fibrosa cystica.

Note: I am indebted to Edward Moore, of the Albany Medical College, for the photomicrographs.

Histologic Findings

The microscopic picture found in those animals which had received a single injection differed considerably from those which had a number of injections. By studying these and the animals which had received multiple injections, four well defined phases were found. The first phase was marked by capillary dilatation, hemorrhage and fresh areas of cellular necrosis. This occurred after any single injection

TABLE II

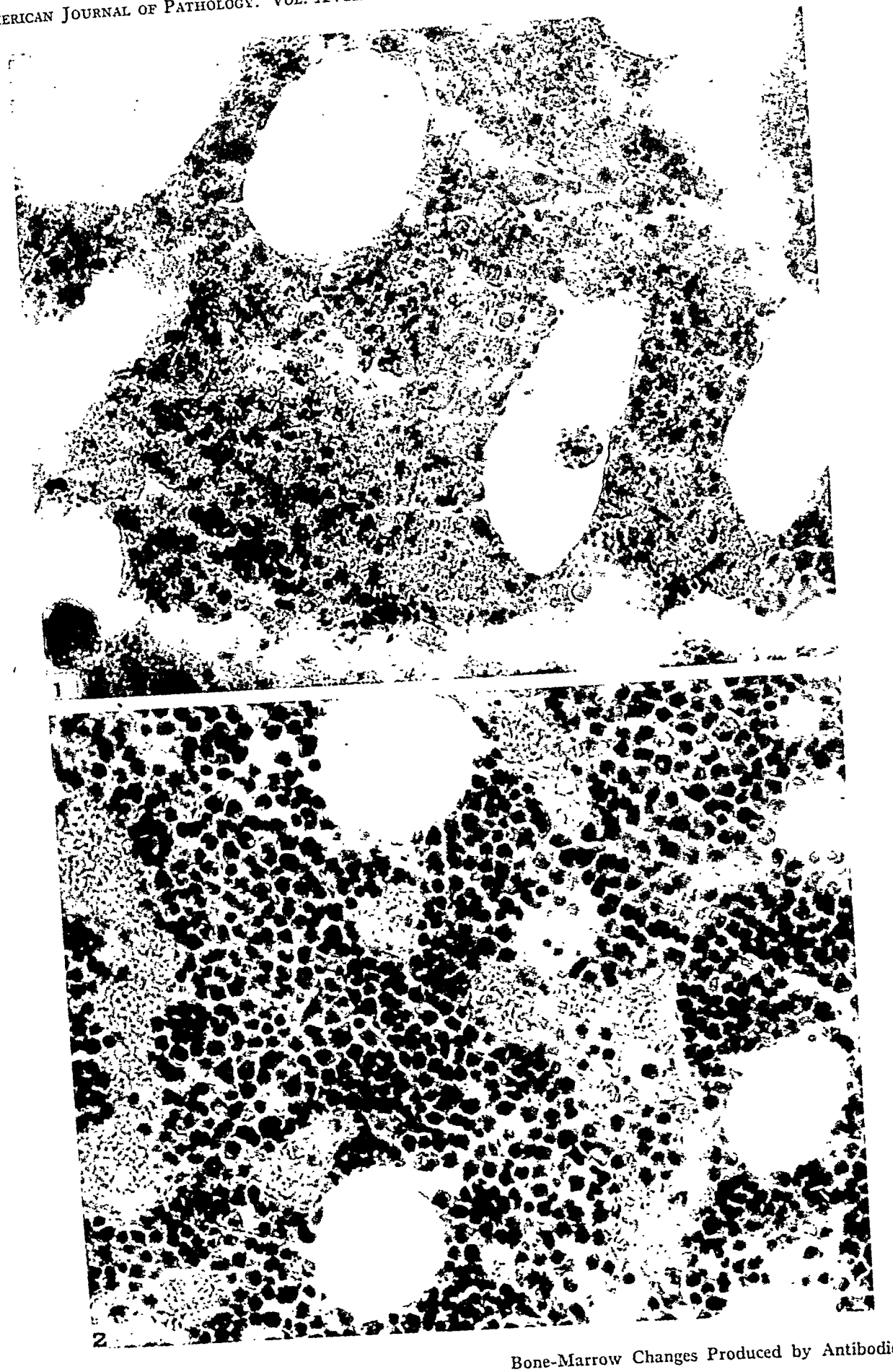
Peripheral Blood Following Repeated Injections

	Days of experiment	Pre-injection count	Amount injected (ml.)	Post-injection count (15 min.)	Hemoglobin
Animal A	1	11,400	10	4,400	
	3	6,150			
	4	10,850			5.5 gm. (36%)
	7	12,650			4.0 gm. (28%)
	9	8,250			3.0 gm. (21%)
	10	9,800	7	6,600	2.8 gm. (20%)
	11	11,650			2.8 gm. (20%)
	15	10,550			5.0 gm. (35%)
	19	7,300	3	7,100	
	21	7,050			
	24	10,950			9.0 gm. (58%)
	28	5,950	3	4,500	
	29	9,300	3	6,050	
	59*	14,400	3		
	61	5,200 (sacrificed)			
Animal B	1*	10,700	2	3,200	
	2	22,400			
	3				
	4	8,800	3	2,100	
	5	8,600			
	7	12,100 (sacrificed)			

* Biopsy.

regardless of whether or not there had been previous ones. The areas of necrosis occurred indiscriminately in all bones studied and involved from a few cells to an area as large as 300 to 500 μ across. All of the cells in the involved area were damaged, although some may have been capable of surviving. Stromal and parenchymal cells were equally damaged. The parenchymal cells showed partial loss of the cytoplasm and poor staining of the remainder, with hyperchromia of the nuclei. Cells in the areas of necrosis in animals receiving multiple injections showed even more irreparable cytoplasmic and nuclear damage (Fig. 1). The second phase was marked by regrowth which quickly passed into the third phase of hyperplasia (Fig. 2). Hyperplastic areas were found in all of the animals receiving multiple injections. Even these

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Bone-Marrow Changes Produced by Antibodies

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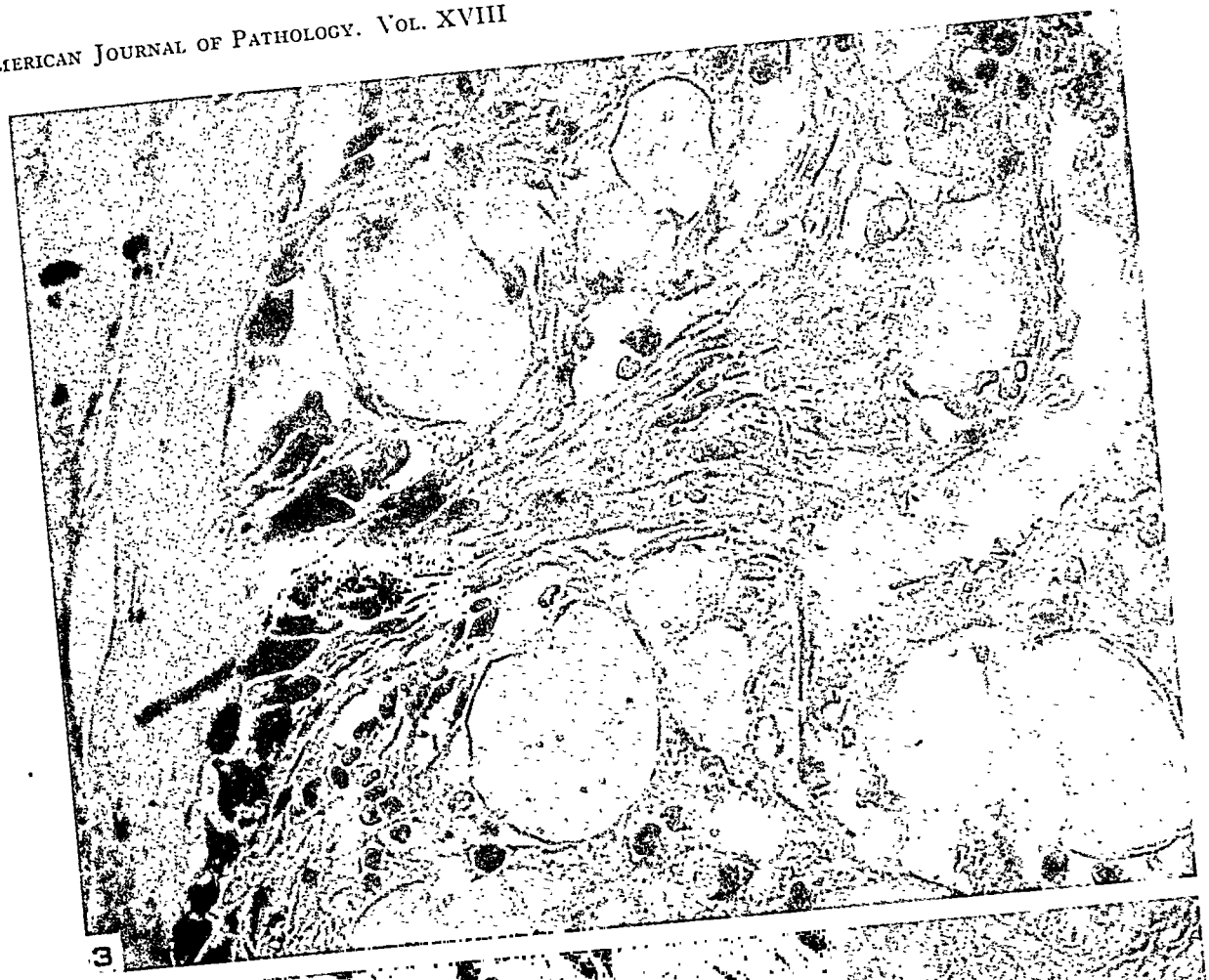
DESCRIPTION OF PLATES

PLATE 106

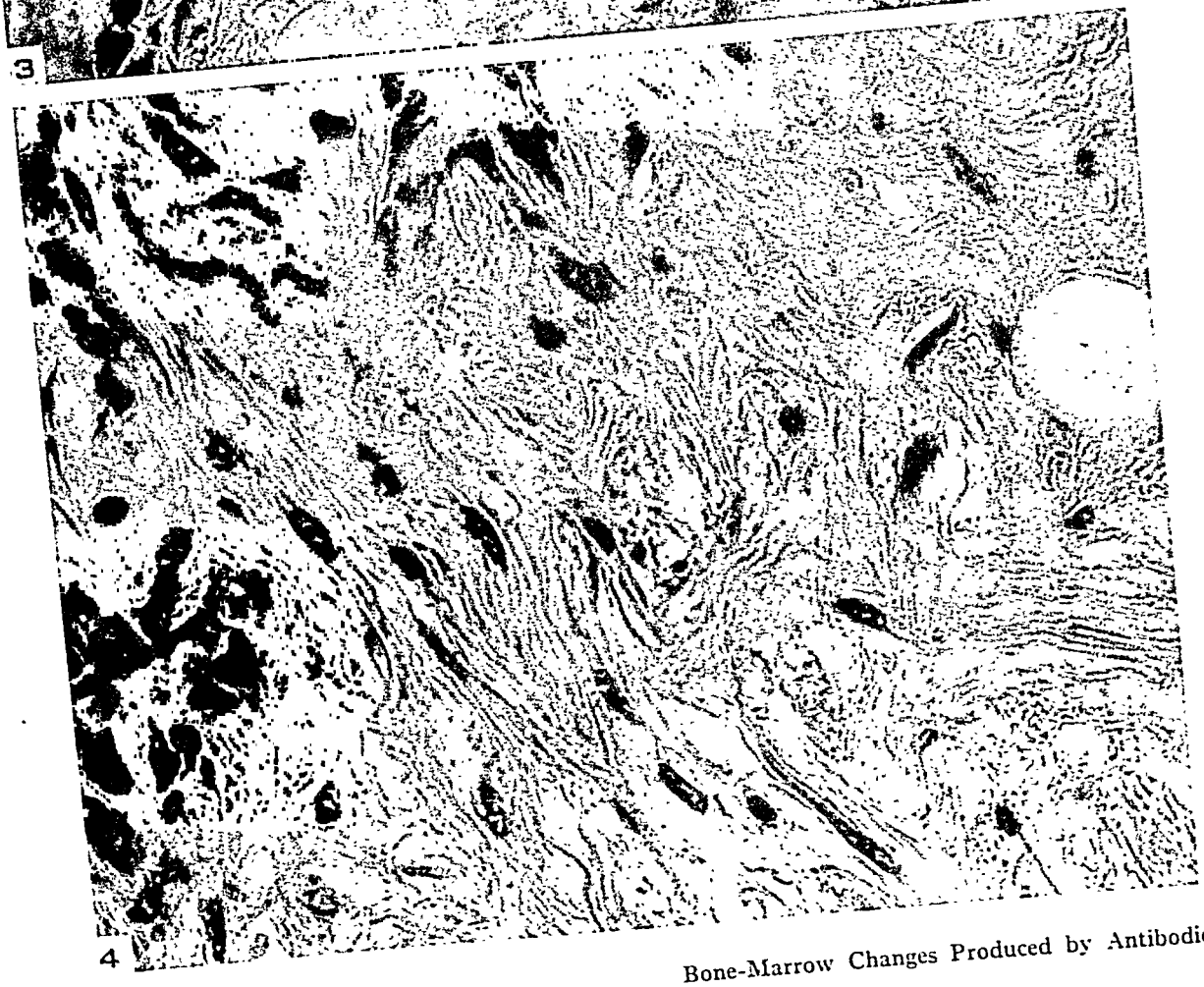
FIG. 1. Pelvic bone marrow from an animal which had received three weekly injections of antibone-marrow antibody. There is widespread destruction of the cytoplasm and understaining of the nuclei. × 540.

FIG. 2. Sternal bone marrow removed from an animal which had received two antibone-marrow antibody injections. There is active hyperemia and extensive, early marrow-cell hyperplasia. × 540.

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4

Bone-Marrow Changes Produced by Antibodies

PLATE 107

FIG. 3. An area from the sternum of an animal which had received three antibone-marrow antibody injections. The large dark-staining cells on the left are thought to be endosteal cells. They lie next to a spicule of bone. × 540.

FIG. 4. An area of extensive, fibrous connective tissue overgrowth. × 125.

KIDNEY LESIONS IN STILLBORN AND NEWBORN INFANTS*

"CONGENITAL GLOMERULOSCLEROSIS"

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(From the Department of Laboratories, Division of Pathology, of the Jewish Hospital of Brooklyn, Brooklyn, N.Y.)

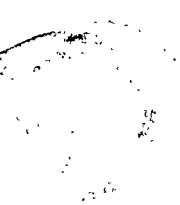
Pathological changes in the kidneys of fetuses and newborn infants have not been the subject of morphological investigation to any great degree in the past. Textbooks and reference works in pathology make little or no mention of such lesions, and the literature contains surprisingly few contributions in this field. Congenital anomalies, uric-acid infarcts, bile-pigment infarcts and tumors of the kidneys are briefly described and dismissed with a few sentences or paragraphs. However, pediatrists and urologists have studied clinically diseases of the kidneys in infants and young children to a greater extent.

In 1908, Karsner [1] described a case of congenital acute nephritis in an infant who died less than 1 hour after birth, and collected three additional cases from the literature. Three cases of neonatal nephritis were described by Conrad,[2] in 1938. Fishberg [3] mentioned having studied pathologically the kidneys of an infant, 4½ months old, who died from subacute glomerulonephritis. Lesions of pyelonephritis have also been described in the newborn by Craig,[4] Helmholz,[5] Hunt [6] and others.

The lesion which constitutes the subject matter of this communication was first described in 1909 by Herxheimer [7] (cited by Gruber [8]). According to him, destroyed, hyalinized glomeruli are found not infrequently in the kidneys of newborns, nurslings and also of older children as the result of developmental defects. His description can hardly be improved upon today. A free translation is as follows:

Hyalinized glomeruli are found in all layers of the cortex but most often near the surface. Sometimes they are grouped about the divisions of an interlobular artery. He found them thirty-eight times in 43 children and felt that further study might also have revealed their presence in the 5 negative cases. The incidence of the lesion did not parallel the severity of the pathological alterations. The earliest change noted by him was thickening of the parietal layer of Bowman's capsule, the result of deposition of fibrous connective tissue lamellae. Hyaline change may not be present at first. When it appears it can be seen, by means of the van Gieson stain, as a red ring of tissue about the membrana propria of Bowman's capsule. Frequently, however, the hyaline material is present only in the form of solitary foci in the

* Received for publication, October 4, 1941.



possible manifestations of a subacute glomerulonephritis. Recently the kidneys from 100 consecutive stillborn, newborn and other infants under 14 months of age were carefully examined for these lesions and 17 more examples were found. Blocks of tissue were fixed in a 4 per cent solution of formaldehyde. Hematoxylin and eosin, Mallory, McGregor and Weigert preparations were made.

Histology of the Glomerulus in the Neonatal Period

Before proceeding with a description of the lesions, review of the histogenesis of the glomerulus in early postnatal life is essential. Gruenwald and Popper [10] recently investigated this subject. The description appended below is taken from their paper:

The glomerulus before birth consists of "an undivided globule covered by a uniform layer of high columnar epithelium which does not extend between the loops. The lumen of the glomerular loops is partly visible and contains a few erythrocytes. . . . The loops are in close contact with each other and no free spaces are left between them. At the vascular pole of the corpuscle the reflection of the visceral to the parietal layer is clearly visible.

"After birth the glomerular loops expand and contain more red cells indicating an increased blood flow. Between the individual loops, clefts are visible which proceed toward the vascular pole, separating 3 to 8 lobules from each other. Parallel to this process changes in the epithelial covering take place. The originally continuous layer of high columnar epithelium is broken up by the clefts. Within the clefts the surface of the glomerular loops is partly covered by epithelium; in other parts no distinct epithelial covering can be seen. The further development leads to a stage in which the almost completely expanded loops show small islands of epithelium on most of their surface. Only on the peak is a continuous layer of high epithelium visible as a remnant of the original visceral layer. Many histological pictures suggest that a part of this high epithelium is cast off during this period, gradually losing its connection with the glomerular loops. This may account for the albuminuria during the first days of postnatal life which is indicated by clotted material in Bowman's space and the tubular lumen. . . . All these changes start in embryonic life in a greater or smaller number of glomeruli. After birth the number of maturing glomeruli increases considerably and the expansion of the capillary loops proceeds rapidly. . . .

"This development is completed within the second year. . . .

"The reabsorptive parts, as Henle's loops and the medullary reabsorptive capillary tufts, are fully developed at the time of birth."

The Lesion

The lesion is found indiscriminately in both kidneys. Grossly the kidneys show no abnormalities and are of average size, shape and weight. Microscopically, the lesion is distributed focally in the cortex of the kidney and is seen most often in the juxta-medullary zone.

In the 13 cases which constituted the original study, the lesion was sufficiently widespread to warrant a tentative diagnosis of "subacute glomerulonephritis" although it was recognized that the process was not a diffuse one. In the control series of 100 consecutive kidneys, 17 showed similar lesions, although not to the same extent. This might

periphery of the tuft. In these instances, he felt that serial sections might have revealed a continuity between the capsule and the tuft. In mild cases, a cleft can be seen between the glomerulus and the hyalinized capsule, the capsular epithelium having been destroyed. Further solution of the affected portion of the glomerulus may take place with gradual hyaline change; or only a sharply circumscribed area of the tuft may be affected, in which case the capsular epithelium may appear distinctly hyperplastic. Adhesions between the capsule and glomerulus are frequently noted. In some glomeruli the hyalinization appears in a more or less ball or pear-shaped form. Completely hyalinized glomeruli are also seen, spindle-shaped cells indicating the capsular boundaries. Should the hyalinization be incomplete, the unaffected portions of the glomeruli may be normal or compressed. Since the capsular portions of the tufts are often lined by rather tall epithelial cells with unusually deeply-staining nuclei, Herxheimer [7] concluded that the loops were retarded in development. Evidence of inflammatory disease was not found in the vicinity of the lesions.

Herxheimer [7] explained the basis of the eventual hyaline formation in the glomerulus by a disturbance in the synchronous development of the epithelial and mesenchymal portions of the malpighian corpuscle. The primary steps are obscure, as it is not known whether the disturbance in the stromal portion antecedes or follows the epithelial irregularity. The former was considered more likely. He saw these structures as "Hamartien" * in the sense of Albrecht, which disintegrate in the subsequent development of the kidney and then disappear.

In 1928, Schwarz [9] studied the kidneys of 80 newborn infants. There was a widespread pneumonic process in 22 infants and in 19 of these, inflammatory and infiltrative changes in the kidneys, in the form of an interstitial nephritis, were found. Lesions of the glomeruli, similar to those described by Herxheimer,[7] were found in 45 of 80 cases, an incidence of 56 per cent. However, in the cases in which inflammatory and infiltrative changes in the kidneys were present, the aforementioned glomerular changes were invariably demonstrable. Before the age of 3 weeks, however, he found the glomerular lesions in barely 30 per cent of his cases. He concluded that the changes were due to the excretion of toxic substances by the kidneys rather than to congenital anomalies, as suggested by Herxheimer.

Materials and Methods

We first noted the lesion as an incidental finding in the course of routine microscopical examinations of the kidneys of 13 stillborn and newborn infants. Some of these lesions had been previously considered

* Hamartien: defects in tissue combination during development (authors' note).

some arterioles no changes were demonstrable, while the glomeruli were the site of the alterations described; especially when the number of lesions was extremely scant and only a few were discovered in a whole section. This may be due to the fact that the vasa afferentia supplying the involved glomerulus did not appear in the section. It is also possible that these arterioles had recovered completely.

These lesions were rarely found in infants above 18 months of age. The altered glomeruli were probably completely hyalinized, leaving a delicate scar which eventually cannot be recognized.

Clinical Features

Age. The average age of the infants whose kidneys were studied was 2.7 months; 78 were less than 3 weeks of age and 22 were more. There were 4 stillborn infants. The oldest child was 14 months of age. The lesion was observed in premature infants as well as in those born at term and in first-born as well as subsequent offspring.

Sex. Among the 30 infants showing the lesion there were 22 males and 8 females, a preponderance of the former in a ratio of 2.7 to 1.

Race. Both white and colored children were affected. The greater number of white babies (24 of 30) is proportionate to the greater number of admissions of white patients.

Symptoms and Signs. There were no characteristic symptoms or signs, nor could any definite clinical syndrome be established. One patient showed anasarca, hydrothorax and ascites, which could be ascribed to renal damage; another showed only edema of the extremities. One infant died in uremia. Other symptoms and signs were referable to accompanying illnesses.

Urinalysis. In ten cases urinalysis was not performed. In the others the results of urinalysis were essentially negative, except for two cases in which occasional red blood cells were noted in the urinary sediment. Five infants showed mild albuminuria, attributable to other causes, such as fever.

Etiology and Associated Pathological Findings

The factors which theoretically may contribute to production of the lesion may be maternal or fetal, or a combination of these.

Concerning maternal factors, nothing definite could be established. There was no evidence or record of renal disease, or toxemia of pregnancy in any of the mothers. There was no high incidence of maternal infections. It is true that some of the mothers had colds during pregnancy, but whether this plays any significant rôle could not be ascertained. Five of the mothers had syphilis; active, latent, or cured. In only two instances did the children born of these mothers show positive serological tests and in only one of these were evidences of congenital

be explained by the fact that an insufficient number of blocks were taken from these kidneys.

The lesion involves essentially the arterioles and the appertaining glomeruli. In the earliest stages there is a proliferation of the endothelium of the arterioles and of the smooth muscle cells of the media (Fig. 1). Concurrently there is an increase in the number of endothelial cells in the tuft (Fig. 2). Their nuclei are large, round or oval, and vesicular; a few are deeply stained. The epithelial cells apparently do not partake in the process at this time and their number remains unchanged. As a result of endothelial proliferation, the lumina of the capillary loops become markedly narrowed or even obliterated. The entire glomerulus becomes ischemic and only occasionally are red blood cells seen within the tufts. At the same time or shortly thereafter, but in some instances preceding the aforementioned alterations, there appears a proliferation of the parietal layer of Bowman's capsule (Figs. 3, 4 and 5). Concentric laminae of spindle-shaped cells with delicate, branching, pink-staining cytoplasm and narrow, elongated and vesicular nuclei appear. These layers, as a rule, are two to five cells in thickness and are rather loosely arranged. It is to be noted that sometimes only the tuft may partake in the process, the parietal layer of Bowman's capsule undergoing no changes. In some instances, the reverse appears to be the case. Not infrequently there is fusion of the tuft with the proliferated capsule, resulting in the formation of "crescents" (Figs. 2 and 3). During this process the walls of the vasa afferentia and arterioles have become progressively thickened and their lumina have become narrowed and even obliterated. Ischemia of the glomerulus is the natural result of such a process. Following this, homogeneous pink-staining material, staining deep blue with the Mallory and McGregor stains, appears within the tuft, or capsule, or both (Figs. 4 to 8). It is apparently deposited between the capillary loops. The deposition of this material may begin peripherally and proceed centrally or vice versa. Only a sector of the glomerulus may be involved and the remainder show no alterations. Concomitantly the number of nuclei of endothelial cells within the corpuscle diminishes until only a few remain. Within the capsule the same process takes place. Ultimately the tuft and capsule may fuse with the formation of a hyaline sphere (Fig. 8). Such occurrences are infrequent, although it is not unusual to see the glomerular tuft represented by a hyaline globule upon which is mounted a single layer of low cuboidal epithelium, separated by a broad or narrow space from Bowman's capsule. The lesion was most frequently seen in the stages of partial hyalinization of the tuft and capsule. Rarely, a few round cells and plasma cells are seen in the interstitial tissue adjoining the affected glomeruli. In

From a consideration of these findings it is quite evident that nothing definite as to the etiology can be concluded. The lesions noted are commonly found as causes of death in infants and there is no preponderance of any single factor.

COMMENT

The significance of the pathological changes in the arterioles and glomeruli described above remains to be determined. They appear to be of no moment where only scattered glomeruli and arterioles are involved. Certainly their presence in 17 of 100 consecutive cases places them almost within the realm of the normal. However, when sufficiently widespread, renal functional damage may occur with the appearance of anasarca or uremia. Whether these lesions play any rôle in predisposing or leading to renal lesions in later life, such as glomerulonephritis or arterial and arteriolar sclerosis, is, of course, only conjectural.

We hesitate to apply any name to the pathological picture described. Until the etiology is ascertained, such a step would be premature. Since the lesions are both proliferative and degenerative in nature, it might be worth while, *pro tempore,* to apply the descriptive term of "congenital glomerulosclerosis" to these lesions. Their focal character possibly may be explained by irregularities in the distribution of functioning glomeruli. This is based upon the belief of some authorities that certain groups of glomeruli or even portions of a single glomerulus function at given periods of time.

These lesions were found by Herxheimer [7] in 88 per cent of the kidneys studied and by Schwarz [9] in 56 per cent. We found them in 30 of 113 cases. This low figure can be ascribed to the small number of sections studied from each case; in some only single sections of each kidney were studied.

Schwarz's [9] observation that the condition is more frequent above the age of 3 weeks has been confirmed. The incidence in infants below this age was 12.8 per cent and above this age, 31.8 per cent. We have been unable to explain this difference in incidence.

In studies upon the renal circulations, Huber,[11] Lee-Brown,[12] Loomis [13] and MacCallum [14] have described atrophic, pathologically altered glomeruli in otherwise normal kidneys (the number increasing with age) and in association with non-glomerulus-bearing blood vessels. It is believed that these vascular and glomerular changes represent involution of renal elements associated with the aging process. These lesions, we conclude, bear no relation to the condition described in this paper.

SUMMARY

1. Certain glomerular and vascular changes in the kidneys of stillborn and newborn infants are discussed.

syphilis found at necropsy. The other child died from hemorrhagic encephalitis following therapy with arsenical derivatives. It is theoretically possible that some maternal factors—toxic, infectious, or hormonal—acting singly or together on the fetus *in utero,* may be responsible for the lesion described. That some of these lesions develop *in utero* cannot be questioned inasmuch as they were observed in stillborn infants. It is possible that some of the lesions of older children may have developed after birth.

As to the child itself, the lesions may be developmental, inflammatory, degenerative or vascular in origin. According to Herxheimer,[7] the frequency of the condition, its occurrence in newborn infants and the absence of evidence of any inflammatory reaction indicated to him that it was probably non-inflammatory in origin. He attributed the condition to retarded development of the kidney. Although Herxheimer made no mention of vascular changes, they were conclusively demonstrated in almost all sections studied by us. Our impression is that alterations in the blood vessels are the significant etiological factors in this condition. What causes these vascular changes is still a problem.

The associated pathological findings may be classified as follows (there is some overlapping, more than one condition having been disclosed at necropsy in many of the infants):

I. Infectious diseases
- Meningitis, 3
- Abscesses of skin, 2
- Sepsis, 2
- Peritonitis, 1
- Otitis media, 3
- Pneumonia, 8
- Empyema, 1
- Acute bacterial endocarditis, 1

II. Anomalies of the heart and great vessels, 5

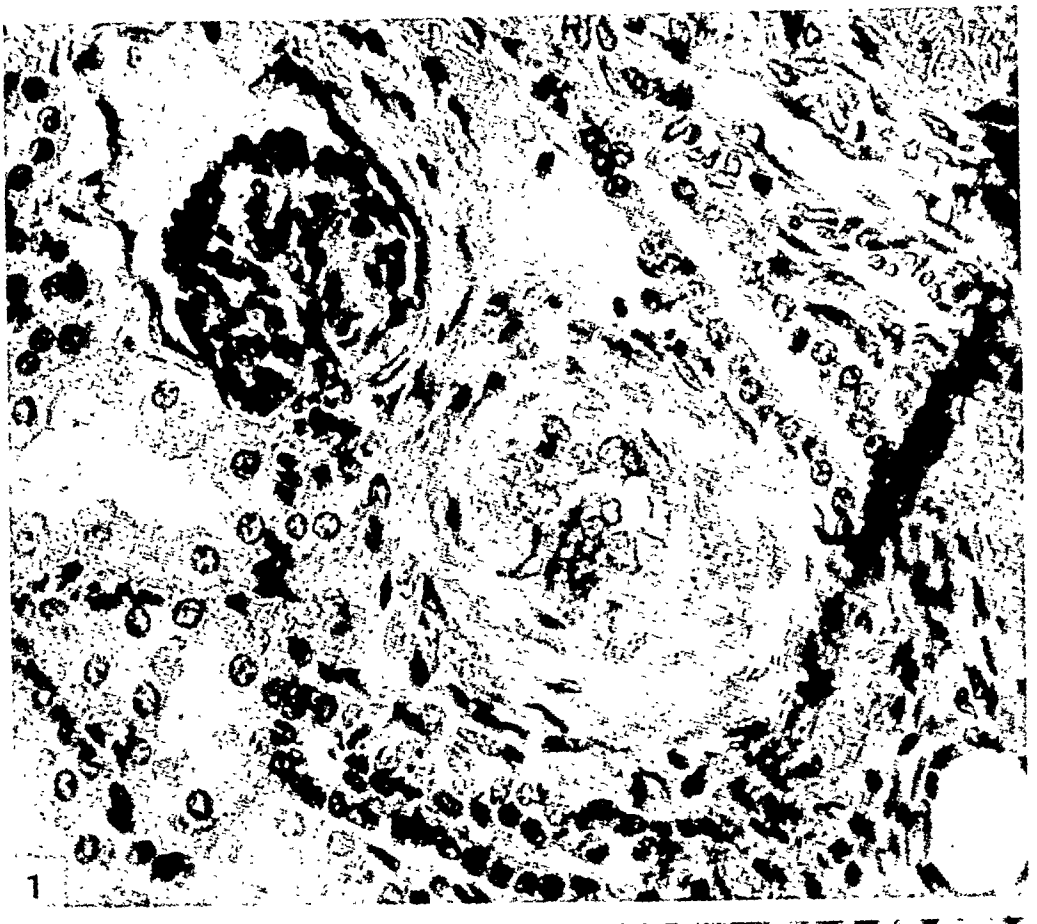
III. Cerebral lesions
- Porencephaly, 1
- Anencephaly, 1
- Hydrocephalus, 1
- Hemorrhagic encephalitis, 1
- Laceration of tentorium cerebelli with hemorrhage, 1

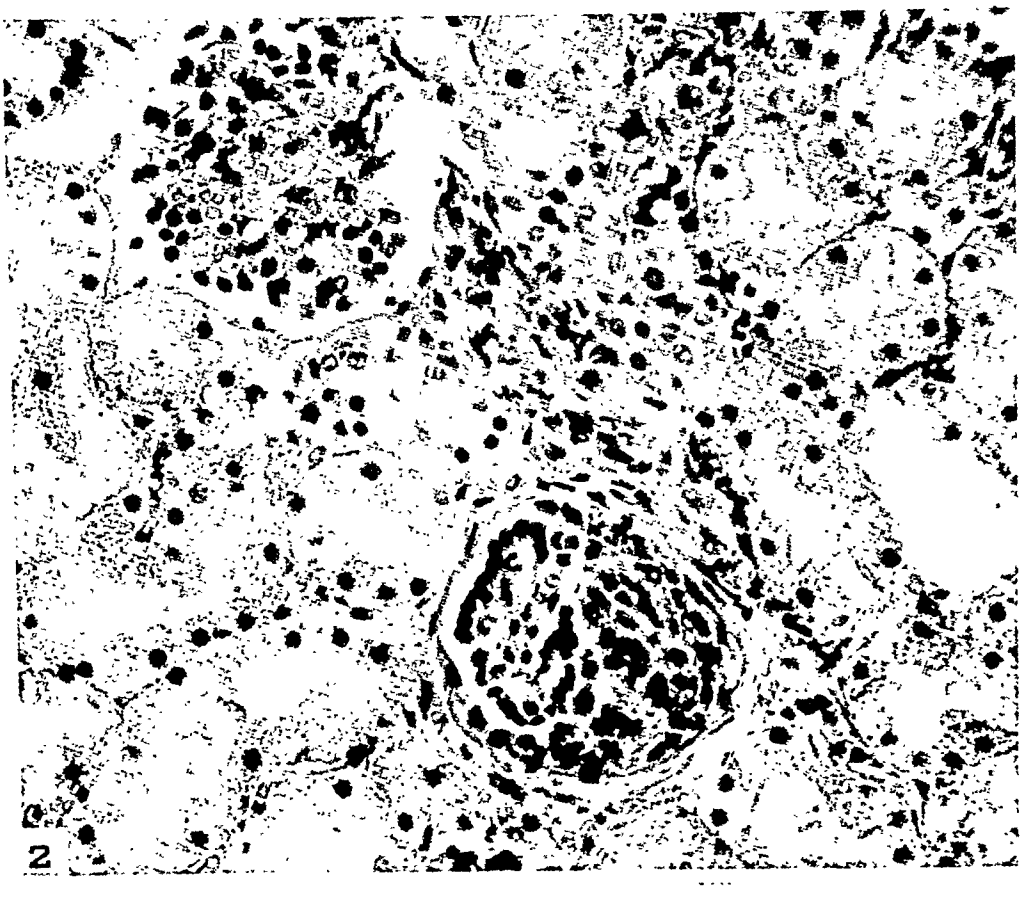
IV. Blood dyscrasias
- Thrombocytopenia, 1
- Leukemic myelosis, 1
- Unexplained widespread hemorrhage, 1
- Erythroblastosis, 1

V. Anomaly of kidney, 1

VI. Metabolic disorders
- Rickets, 2

VII. Obstetrical abnormalities
- Prematurity, 2
- Breech presentation, 1
- Difficult labor, 1
- Premature separation of placenta, 1





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2. The lesions involve the arterioles and their appertaining glomeruli and are associated with hyaline changes in the tufts and capsules. They are bilateral and focal in distribution and are recognizable only by microscopical examination.

3. They are not associated with any clinical syndrome nor is their etiology or significance understood, although they are congenital and may be of vascular origin.

4. It is suggested that this lesion be termed congenital glomerulosclerosis.

NOTE: The authors acknowledge with thanks valuable suggestions in the preparation of this paper from Dr. Jean Oliver, Professor of Pathology, Long Island College of Medicine.

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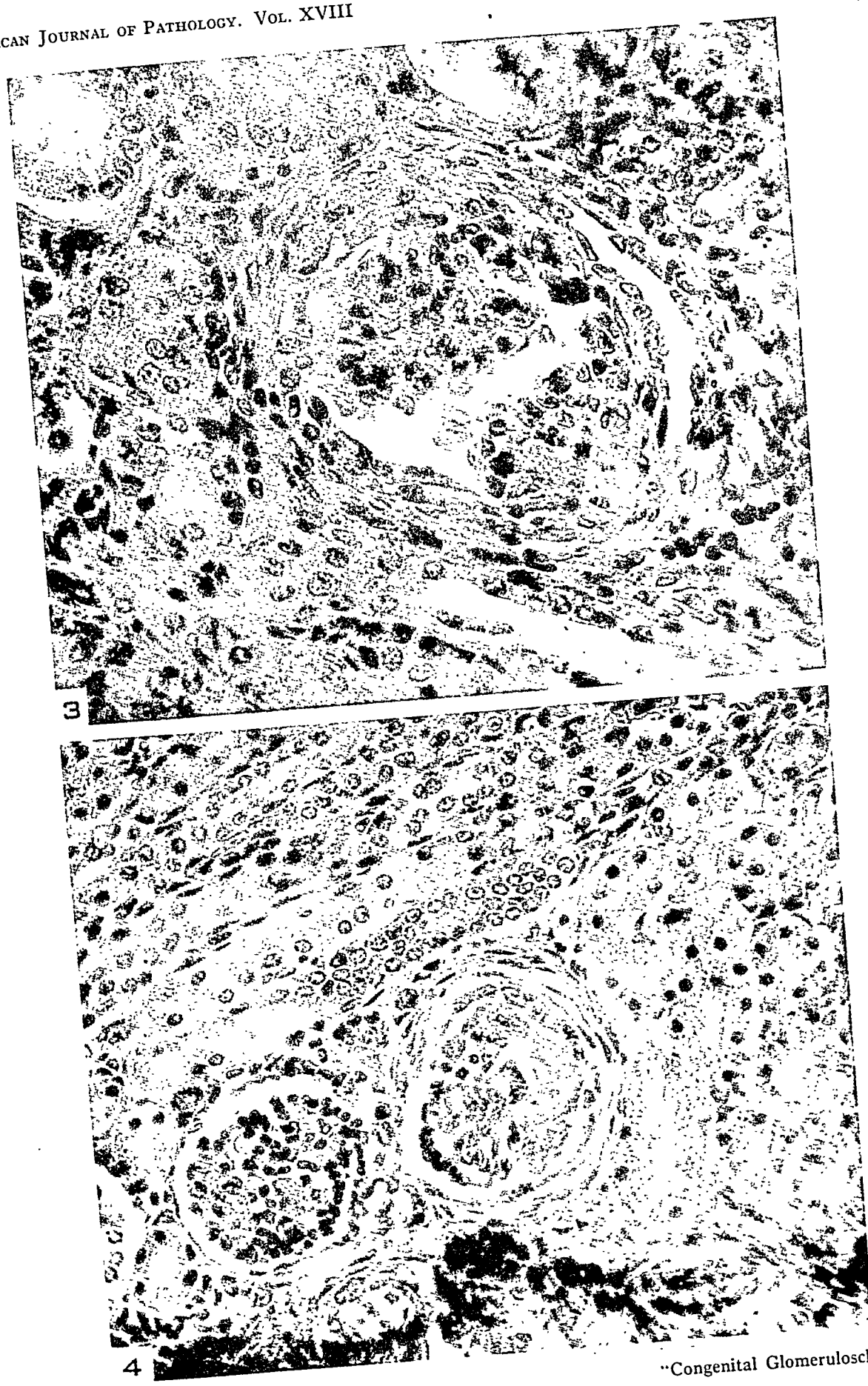
DESCRIPTION OF PLATES

PLATE 108

FIG. 1. Infant, aged 2 months. Arteriole with thickened wall, hyperplasia of endothelium and narrowing of lumen. Hematoxylin and eosin stain. × 600.

FIG. 2. Infant, aged 4 months. Endothelial and epithelial proliferation with "crescent" formation. Hematoxylin and eosin stain. × 350.

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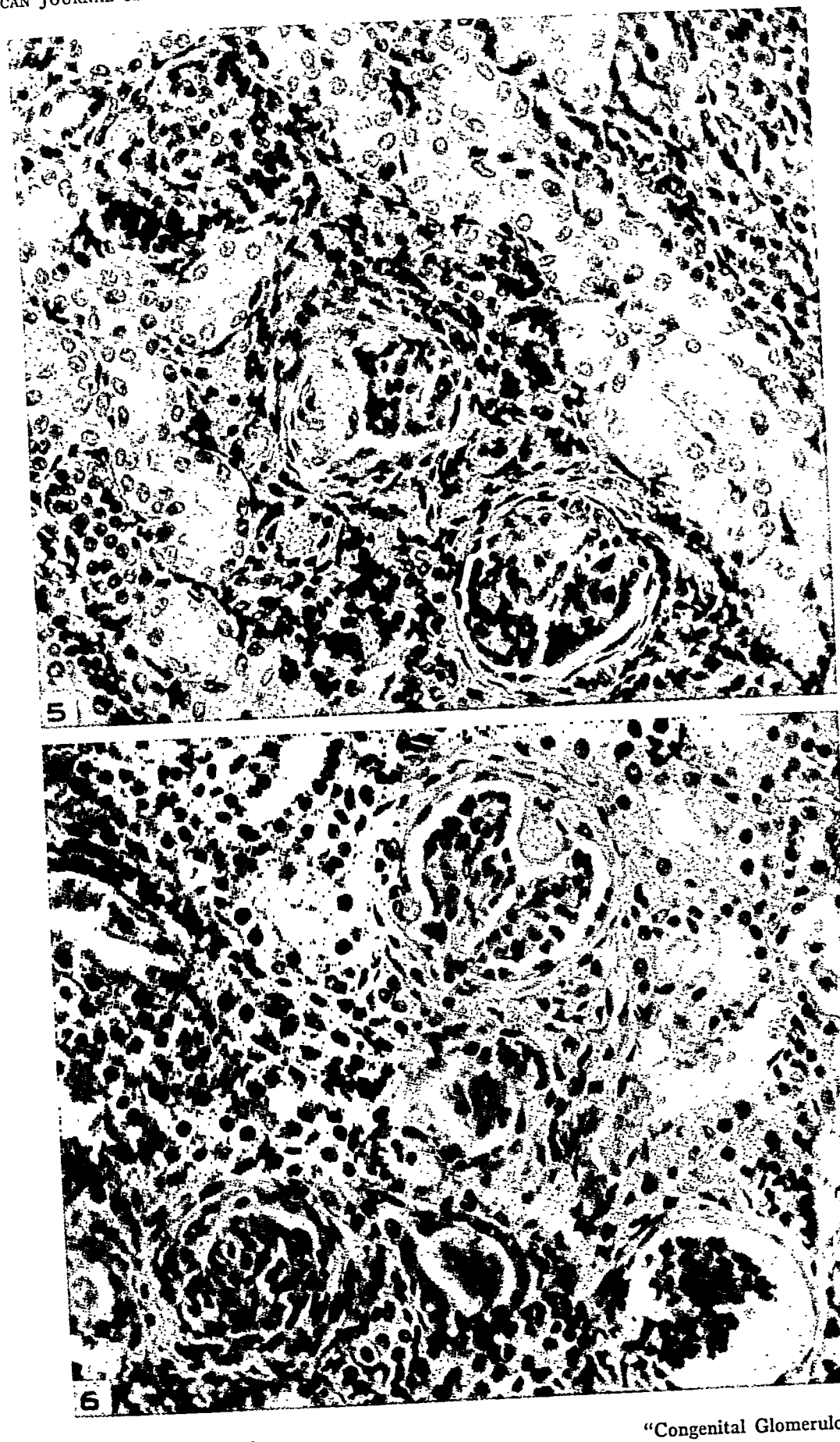
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PLATE 109

FIG. 3. Infant, aged 2½ months. Later stage than that of Figure 2 with marked proliferative changes, fusion of tuft and capsule with almost complete obliteration of subcapsular space. Hematoxylin and eosin stain. × 900.

FIG. 4. Stillborn infant. Beginning hyaline formation with slight capsular proliferation. Hematoxylin and eosin stain. × 525.



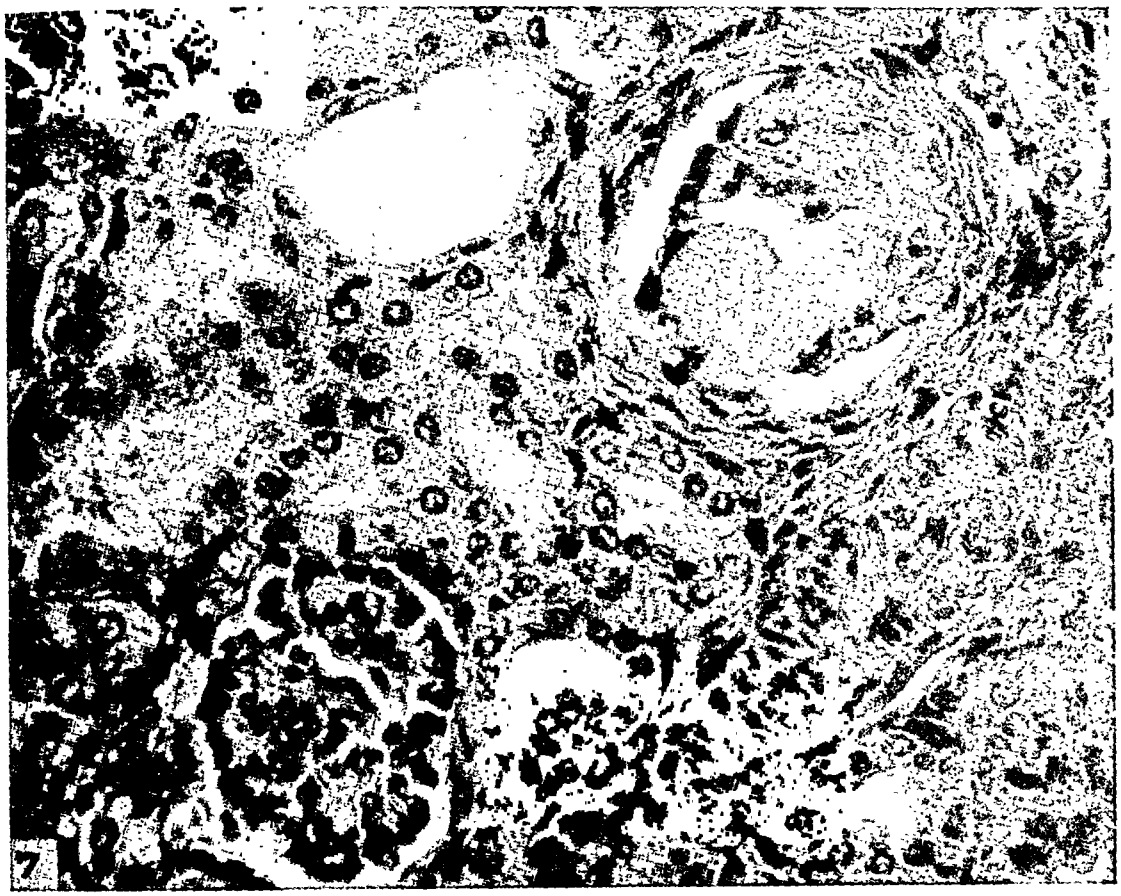
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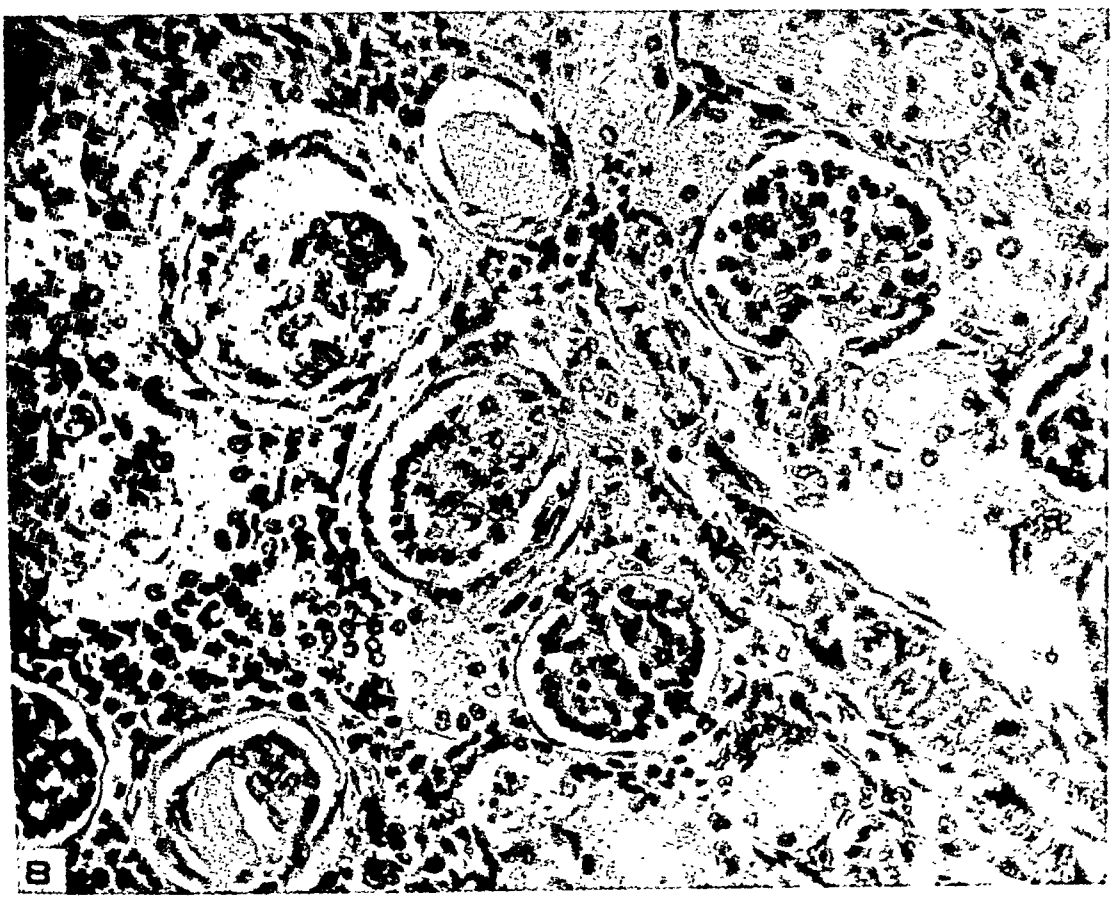
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PLATE 110

FIG. 5. Infant, aged 3 weeks. Fusion of tuft and capsule, early hyaline changes and intraglomerular clefts. Hematoxylin and eosin stain. × 525.

FIG. 6. Infant, aged 12 months. Fusion of tuft and capsule, early hyaline changes and intraglomerular clefts. Hematoxylin and eosin stain. × 525.





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PLATE III

FIG. 7. Infant, aged 6 months. Advanced hyaline changes; thickening of parietal layer of Bowman's capsule. Hematoxylin and eosin stain. × 575.

FIG. 8. Infant, aged 10 days. Late lesion with partial to complete replacement of tuft by hyaline material. Hematoxylin and eosin stain. × 440.

CHLOROLEUKEMIA *

Report of a Case with Special Reference to Its Neoplastic Nature

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That chloroma or chloroleukemia is a rare disease is sufficiently proved by the small number of cases published since the first report of Burns [1] in 1823. Kandel [2] estimated that up till 1936, 175 undubitable cases had been reported. We have found 15 additional cases in the literature available to us: the cases of Nicod,[3] Marten and Meyer,[4] Apitz [5] (2 cases), Dustin and Thomas [6] and Jones [7] in the original and those of Laurie,[8] Zanela,[9] Roehm, Riker and Olsen,[10] Horsfall,[11] Barsoum,[12] Zemplén,[13] Frost [14] and Conti [15] in abstracts. (The case of Foulon and Desclaux [16] cannot be considered as chloroma, the green color not having been observed.)

It is now generally agreed that chloroma is a variant of myelogenous leukemia (Forkner[17] (*loc. cit.*, p. 164), Kandel[2]) in which the leukemic cells show a more marked invasiveness than in the ordinary forms of myelogenous leukemia and a stronger tendency to the formation of tumors, the color of which has given the disease its name. It is quite obvious that these qualities have contributed much to the conception that chloroma and the leukemias are neoplastic in nature. This opinion, already expressed by several authors, has recently received much emphasis from animal experiments and from observations on human leukemia, especially those of Apitz.[5, 18] The number of cases of chloroleukemia and myelogenous leukemia, published with this problem in view, *i.e.*, the relationship of leukemia to the neoplastic diseases, is still small, and so we feel justified in reporting a case of chloroleukemia in which the invasive character of the leukemic cells was very pronounced.

Report of Case

HISTORY

A colored male, native of Trinidad (B. W. I.) and 41 years old, was sent to the hospital by his physician with the following history: The patient was seen by him for the first time 8 months previously. He then complained of a swelling on the upper part of the sternum, which had grown slowly. The tumor had the size of a small fist, was moderately firm and adhered to the bone and overlying skin. As the Wassermann and Kahn reactions were strongly positive, a diagnosis of gumma was made and antisyphilitic therapy instituted. After being treated for 1 month, the patient stated that he felt very well and did not want further treatment. He was

* Received for publication, November 8, 1941.

Microscopical Examination

The tissues were fixed in Heidenhain's corrosive sublimate-formaldehyde mixture (saturated aqueous solution of sublimate, two parts; water, two parts; 35 per cent formaldehyde solution, one part) and in a 7 per cent aqueous solution of formaldehyde. They were embedded in tissuemat after treatment with methylbenzoate-celloidin.

Sections were stained with hematoxylin and Giemsa's stain; orange, eosin and toluidin blue; iron hematoxylin and picrothiazine; hematoxylin and orcein; azocarmine and aniline blue, and Masson's tetrachrome stain according to the modification of Larson and Levin.[19] For the peroxidase reaction the method of Sato was used on frozen sections.

General Description of the Leukemic Cells. The leukemic cells had a size two or three times that of an erythrocyte; the shape was round, oval or more irregular, due to reciprocal pressure and adaptation to the available space; the cells were in general distinctly outlined. The nuclei were relatively large with a sharp nuclear membrane and stained lightly; they were in general round or oval, but in many cells more or less deeply indented, sometimes on both sides. The nuclei contained small chromatin granules and always one or two conspicuous nucleoli. The protoplasm was basophilic, although not so strongly as that of plasma cells or as the basal part of the acinar cells of the pancreas, in which organ this difference in staining reaction can be easily observed. In sections stained with hematoxylin and Giemsa's stain sometimes very small, pale red rods or granules or a small pink-colored area were found in the protoplasm. Basophil, eosinophil or neutrophil granules were entirely absent. The peroxidase reaction was negative. Mitotic divisions were numerous.

Pectoral and Intercostal Muscles. In the pectoral and intercostal muscles the invasive properties of the leukemic cells could be most easily observed. The muscle fibers were separated by large collections of leukemic cells and were often atrophic. In other places they were still in more or less close contact and were apparently normal. Many fibers were invaded by the leukemic cells, and in such a way that the peripheral sarcoplasm, with its nuclei and fibers, formed a hollow and sometimes thick-walled cylinder, in the lumen of which the leukemic cells were found, and, as proved by the presence of mitotic divisions, were proliferating. Sometimes the cells were so closely packed that the cell borders were indistinct. The fibers in the walls of the cylinders were easily demonstrated in cross and longitudinal sections and in the latter the cross striations were clearly visible, especially when the Masson stain was used. On longitudinal sections, sometimes thin strands

not seen again till the day of admission. He then appeared desperately ill, was very anemic, and voided bloody urine. His skin showed many hemorrhages. The tumor had grown somewhat in size.

The patient died a short time after admission.

REPORT OF AUTOPSY

Gross Examination

Autopsy was performed 20 minutes after death. The most important findings were as follows: There was general anemia and in the skin many hemorrhages, sometimes 2 cm. in diameter, were found. The peripheral lymph nodes were only slightly enlarged. On inspection and palpation the head showed nothing abnormal. The tumor on the manubrium sterni had the size of a man's fist, was moderately firm, adhered to the skin and the bone and infiltrated the pectoral muscles, especially on the right side. The center of the tumor was necrotic. The color of the neoplastic tissue was olive-green. In the periosteum of several ribs green tumor-tissue, which infiltrated the intercostal muscles, was found. The sternal bone was partly eroded by the tumor; the sternal marrow was also green. A large green tumor was found in the upper mediastinum; the tumor was attached to the manubrium sterni and infiltrated the upper lobes of both lungs. The pleural lymph vessels on both sides were very conspicuous and offered the same picture as in lymphangitis carcinomatosa, the only difference being that their color was green. The hilus nodes were swollen and greenish gray on section.

In the pericardium a few small, green nodules were observed; the heart was normal, except for a few petechiae in the epicardium. In the pleura diaphragmatica and in the peritoneal covering of the abdominal wall small, flat, green tumors were seen.

The spleen was enlarged, weighing 350 gm., and normal in consistency. On section the color was dark red, the trabeculae were small and the malpighian corpuscles not visible. The liver was enlarged, weighing 1900 gm. On section its color was pale red-brown. In the body of the pancreas a green tumor, measuring 1 by 3 cm., was found; the peripancreatic lymph nodes were swollen. A green tumor was situated in the cortex of the left kidney; the tumor extended into the medulla; the left renal pelvis was filled with blood clots. The marrow of the vertebrae was pale gray-red. Examination of the skull and of the brain was not possible.

Now, after preservation in formaldehyde solution for 2 years, the green color of the tumors, although somewhat faded, is still visible.

shown by the connective tissue stains; the walls of the venous sinuses were normal. The malpighian corpuscles were small. In the sinuses and in the pulpa reticulum many leukemic cells were found. The cells were not closely packed; mitotic divisions were frequent. In some sinuses large macrophages, loaded with hemosiderin, were seen. Only after prolonged searching could a few polymorphonuclear leukocytes be found.

Liver. The capillaries and the periportal connective tissue contained only a few leukemic cells, some of which were dividing. Many "triangles" were entirely free from leukemic cells.

Pancreas. Sections taken from the border of the green tumor showed important changes. The pancreatic tissue was densely infiltrated by leukemic cells and the acini were atrophic or had completely disappeared. In the atrophic acini the acinar cells still contained zymogen granules. Many islands were still intact and very conspicuous, and their different cell types were easily recognizable. In some places the invasion of islands by leukemic cells could be observed. In general the leukemic cells were lying in the meshes of an amazingly rich network of thin, branching fibers and were actively proliferating. They also infiltrated the neighboring fat tissue.

Kidney. Over the green tumor the capsule was thickened and contained many leukemic cells. In structure the capsule resembled the infiltrated pleura. The cortex was heavily infiltrated; the renal parenchyma had almost completely disappeared, only a few glomeruli and atrophic tubules remaining. The leukemic cells were closely packed and showed many mitotic divisions, which, however, were not evenly distributed.

Heart. In the epicardial fat and between the muscle fibers the leukemic cells were present in small collections; on the contrary, enormous masses of leukemic cells were found in the ventricles caught between the trabeculae.

Lymph Nodes. The peripancreatic lymph nodes were invaded from without, the marginal sinus being full of leukemic cells which from there penetrated the tissue of the gland. In the hilus nodes of the lungs the lymphatic tissue was almost completely replaced by leukemic cells. True myeloid metaplasia could not be observed.

Bone Marrow. The vertebral marrow was very cellular; however, only relatively few normal cells were found, mostly nucleated red cells and eosinophilic myelocytes and leukocytes. Neutrophilic cells and megakaryocytes were rare. The other cells, found in the marrow, were leukemic cells.

Blood Vessels. Apart from the blood vessels in the lungs, vessels

of protoplasm, recognizable in Masson-stained sections by their red color, could be seen running through the masses of leukemic cells from one side of the cylinder to the other, so that the lumen seemed to be divided into small compartments. As this could be observed also in cross sections of the muscle fibers, it cannot be due to tangential sectioning.

When the number of leukemic cells increased, the walls of the cylinders became thinner and only a very thin layer of sarcoplasm remained, in which, however, the fibrils persisted in many cases. The Masson stain was again the most useful for the demonstration of these last remnants of the muscle fibers. The next and last stage was the complete disappearance of the muscle fibers. In several places, where the muscular tissue had been completely replaced by leukemic cells, intact muscle spindles were found.

Lungs. The pleura of the upper lobes was much thickened and the leukemic cells were found lying between thick bundles of collagen, from which finer bundles and thin fibers were branching off. Often the leukemic cells were surrounded by very fine fibers, which stained red with picrothiazine. The lymph vessels of the pleura were distended with closely packed leukemic cells. In the lungs, infiltration was very heavy in the interlobular septa, which were greatly thickened, and around the bronchi and greater blood vessels, which were surrounded by enormous masses of leukemic cells. Many mitotic divisions were found here. The walls of the bronchi were invaded in many places and the leukemic cells were lying just below the bronchial epithelium. This epithelium had completely disappeared in some places and here the leukemic cells were found in the bronchial lumen. Invasion of the walls of the blood vessels was also observed, the leukemic cells proliferating between the media and the endothelium, which was pushed towards the lumen. Sometimes the smaller blood vessels were filled with leukemic cells, some of which were dividing, so that these vessels could not be distinguished from vessels filled with tumor thrombi. Dividing leukemic cells were also found in vessels containing but a few cells. Where the intrapulmonary lymphatics were still recognizable, they were filled with leukemic cells. The alveolar septa were thickened by infiltration with leukemic cells, which were also found within the capillaries. The "alveolar cells" were swollen and in the alveoli huge collections of leukemic cells, together with large vacuolated, often dust-loaded macrophages, were seen. In the alveolar lumina the leukemic cells were proliferating, as proved again by the presence of mitoses. Polymorphonuclear leukocytes were entirely absent.

Spleen. The structure of the spleen had remained intact as was

nodes showed on section a distinctly green color, and in the left retroperitoneal space a large, partly necrotic, yellowish white tumor was found. This tumor infiltrated the muscles and was composed of myeloblasts and myelocytes, part of the latter containing eosinophilic granules. The cells proliferated inside small venules, formed tumor thrombi and infiltrated the neighboring fat tissue. Here even megakaryocytes were seen. In case no. 2 a large hemorrhagic tumor was found between the muscles of the thigh, composed of myeloblasts and myelocytes and infiltrating the muscles. It is of special interest that in this case the leukemic cells proliferated inside empty sarcolemma tubes and the author emphasized the fact that he considered the sarcolemma as previously emptied by necrosis of the muscle fibers. In case no. 3 the paratracheal and the right cervical lymph nodes showed a greenish color and here the leukemic cells showed a special aggressiveness and infiltrated the muscles and the wall of the jugular vein.

When we compare these findings of Apitz [5,18] with the anatomical and histological data of our case, we cannot fail to observe that, although the topographical localization of the lesions is different, a very great similarity exists. We, too, found invasion and destruction of the walls of blood vessels, the formation of tumor thrombi, the destruction of the bronchial wall and of the parenchyma of the pancreas and kidney; the invasion of the muscles could be observed even better than in the second case of Apitz. We could show that the muscle fibers themselves were invaded by the leukemic cells, just as the muscle fibers of the tongue are invaded by a lingual carcinoma and the pectoral muscles by a carcinoma mammae. The changes in the pleural lymphatics were the same as in the so-called lymphangitis carcinomatosa (Kaufmann [33]), the only difference being that the lymph vessels were filled with leukemic cells and that their color was green. Finally, the rapid proliferation of the leukemic cells could be proved by the presence of numerous mitotic divisions.

Were it not for the fact that *normal* blood cells are capable of infiltrating other tissues, perhaps few investigators would doubt the blastomatous nature of such cases as they show all the features considered necessary for the diagnosis of a malignant tumor. In connection with this we first wish to point out that, although invasiveness is an inherent property of the chorionic epithelium, nobody seems to doubt the neoplastic nature of chorio-epithelioma; and, secondly, that it is not sufficiently proved that *normal* myeloblasts from non-leukemic men and animals can invade other tissues in the same way as mature leukocytes in inflammation and the *abnormal* myeloblasts in leukemia.

A much used argument against the neoplastic nature of the leukemias

in other parts also were invaded, especially the veins in the pectoral muscles, the walls of which were destroyed by the leukemic cells, only a few smooth muscle fibers being recognizable in Masson-stained sections.

Discussion

The color of the tumors and infiltrations is in itself sufficient for the diagnosis of chloroma; however, as blood counts and the study of blood films were impossible, the evidence for the diagnosis of myelogenous leukemia must be gathered from the anatomical and histological findings. The almost complete absence of polymorphonuclear leukocytes from the different organs and from the blood; the fact that in the lumina of the blood vessels only the cells, previously described as leukemic, were found, and the extensive replacement of the bone marrow by these cells constitute, in our opinion, sufficient proof. The slight histological changes found in the liver are not so typical for ordinary leukemia, but occur often in chloroma (Dustin and Thomas,[6] Kandel,[2] Nicod [3]). We believe also that the cytological details of the leukemic cells leave little doubt as to their nature. The size of the cells, the structure of their nuclei with the fine chromatin granules and the large nucleoli are much more typical for undifferentiated myelogenous cells or myeloblasts than for lymphocytes and their parent cells. When we compare the cells found in our case with those described and depicted by Forkner [20] in monocytic leukemia, this disease can be easily excluded. We cannot attach much importance to the fact that in our case the peroxidase reaction was negative. Premature bone-marrow cells may show a negative reaction [Piney,[21] Downey,[22] Jaffé,[23] Forkner [17] (*loc. cit.*, p. 51)] and it is also possible that the long preservation of the tissues in formaldehyde solution (18 months) was responsible for this result.

As stated in the introduction, the conception of the leukemias as neoplastic in nature (Babes,[24] de Vries,[25] Lignac [26] and others) has received much support from experimental studies on leukemia in rodents (Snijders,[27] Tio Tjwan Gie,[28] Furth,[29] Hall and Knocke,[30] Murphy and Sturm [31]). However, as some authors seen to suggest that human leukemia and the transmissible leukemias of rodents are not analogous (Israëls [32]), we must turn to the analysis of human cases and here the observations of Apitz [5,18] are of special interest. In two papers he described twelve cases of human leukemia, offering much evidence for the conception mentioned above. Three of his cases were myelogenous leukemias and these showed the following features: In case no. 1 the cervical, tracheal, perigastric and retroperitoneal lymph

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is the fact that in most cases no tumors can be found. This, however, can also occur in diffuse gliomatosis of the leptomeninges (Connor and Cushing [34]), which is considered a truly neoplastic process. The opinion expressed by Kandel [2] that the myeloblasts, which are already showing a rapid and disorderly proliferation, simply assume another rôle in chloroma and become capable of destructive invasion, is in contradiction with the modern conceptions of malignant tumors.

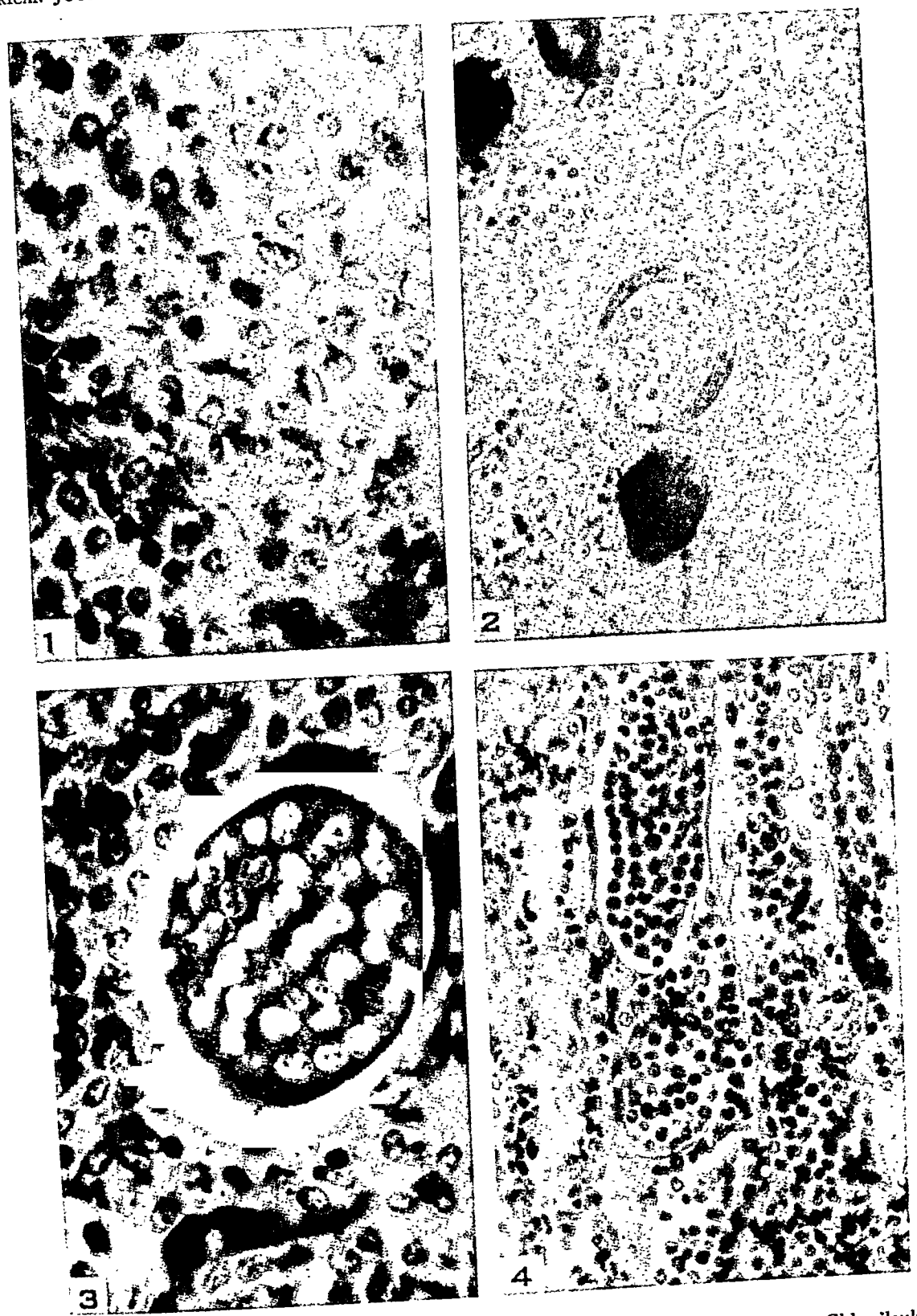
When, finally, we compare the definition of a tumor given by Ewing,[35] "a tumor is an autonomous new growth of tissue," with Forkner's [17] (*loc. cit.*, p. 1) definition of leukemia, "an invariably fatal systemic disease of unknown etiology, primarily involving the blood-forming organs and characterized by widespread, rapid, and disorderly proliferation of the leukocytes and their precursors," we see that the most important difference lies in the expression "systemic disease." It should, however, be remembered that the conception of leukemia as a systemic disease originates from a time when far less was known about tumors and the leukemias themselves and was for many years carried on through medical, especially clinical, literature, without adequate proof being offered.

Summary

A case of chloroleukemia, occurring in a colored male, 41 years old, is described. The leukemic cells were undifferentiated myelogenous elements, which invaded muscle fibers, blood vessels, lymphatics and other tissues in the same manner as tumors generally recognized as malignant. The relationship between the leukemias and the neoplasms is discussed briefly and the similarity of the two processes emphasized.

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DESCRIPTION OF PLATES

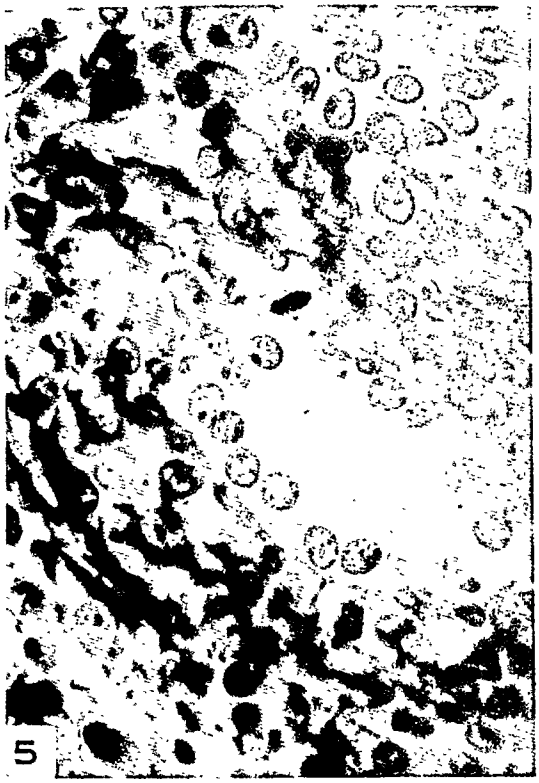
PLATE 112

FIG. 1. Leukemic cells. × 750.

FIG. 2. Transverse section of the pectoral muscle. The leukemic cells are seen lying inside and between the muscle fibers. In one muscle fiber three mitotic divisions are seen. × 357.

FIG. 3. From a section of the pectoral muscle. The muscle fibrils are visible. × 750.

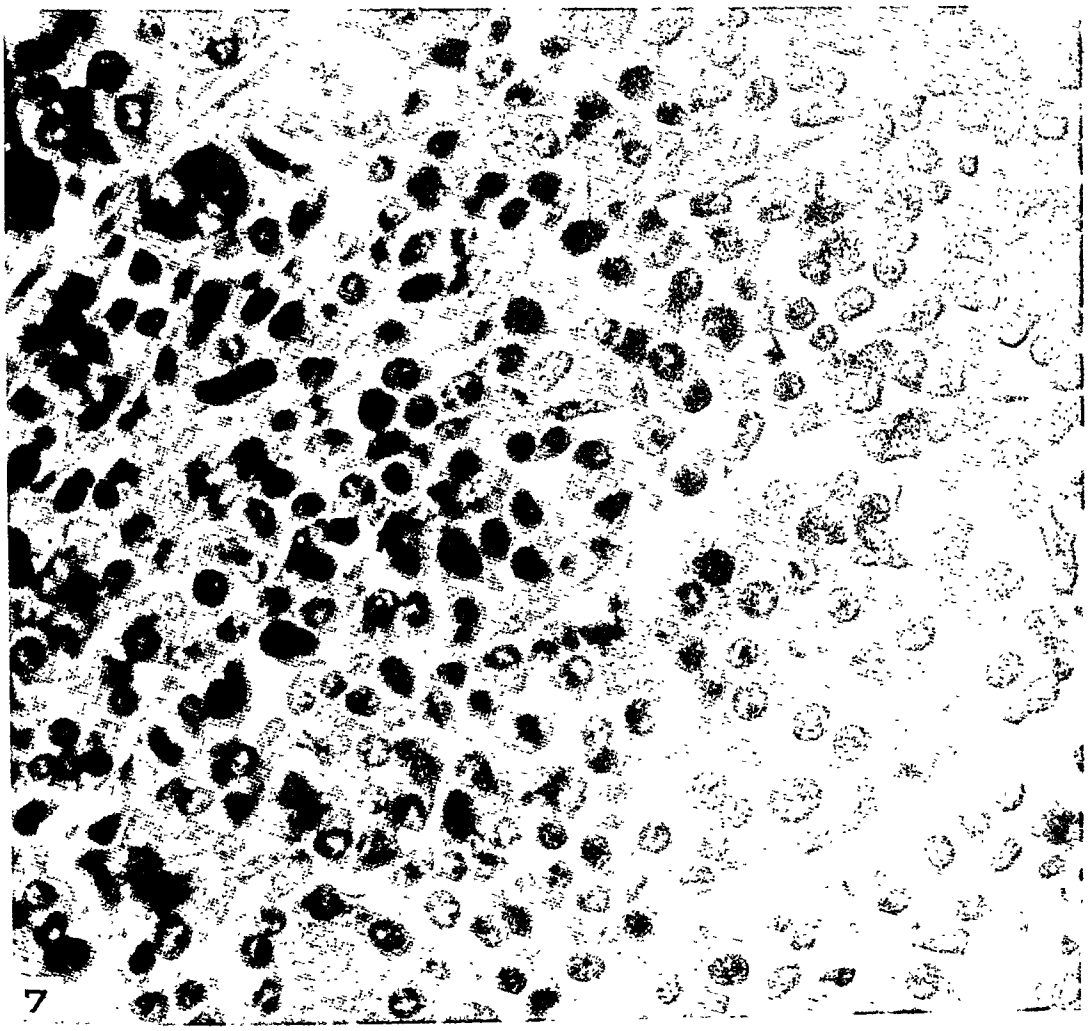
FIG. 4. Longitudinal section of muscle fibers infiltrated by leukemic cells. The hollow cylinder seems to be divided into small compartments. × 357.



5



6



7

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PLATE 113

FIG. 5. Small pancreatic duct surrounded by leukemic cells. There is one mitotic figure in the field. × 750.

FIG. 6. Pancreas. The beginning infiltration of an islet is shown. × 715.

FIG. 7. Renal cortex with a heavy infiltration of leukemic cells. × 357.

THE HEART IN UREMIA *

CYRIL SOLOMON, M.D., J. EUGENE ROBERTS, M.D., and JAMES R. LISA, M.D.
(From the Pathological Laboratory, City Hospital, Welfare Island, Department of Hospitals, New York, N. Y.)

This communication is a report of the pathological findings in the hearts of 50 patients dying while in uremia. Acute endothelial hyperplasia of the coronary arterioles, producing narrowing or thrombosis, was present in patients with renal necrotizing arteriolitis. With this one exception, there were no cardiac changes characteristic either of the uremic state or of the associated renal pathology.

The selection of the material rested upon the clinical diagnosis of uremia and the histological demonstration of kidney damage compatible with complete renal decompensation. The criteria for the diagnosis of uremia were an azotemia of not less than 90 mg. of blood nonprotein nitrogen, urinary abnormalities and coma. Prerenal azotemias were eliminated from consideration. The pathological classification of the kidneys was the same as that adopted in another study.[1]

The kidneys were classified in five groups. There were 18 cases of pyelonephritis, 10 of nephrosclerosis, 8 of necrotizing arteriolitis, 4 of acute diffuse glomerulonephritis and 9 of chronic diffuse glomerulonephritis. All kidneys with necrotizing arteriolitis had a greater or less degree of nephrosclerosis.

The weights of 49 hearts were recorded. They ranged from 175 to 940 gm. One heart which was not weighed was noted as hypertrophied. The smallest heart was from a child of 9 years with chronic diffuse glomerulonephritis; the myocardium was definitely hypertrophied. As can be seen from Table I, the widest ranges were found in pyelonephritis and nephrosclerosis. The greatest weights tended to occur with renal necrotizing arteriolitis.

Acute diffuse fibrinous pericarditis was present in 7 cases. An acute fibrinous pericarditis limited to the base of the heart and the intrapericardial portions of the large vessels was present in 5 others. Chronic adhesive pericarditis, in one instance of rheumatic nature, was found in 6. Although the fibrinous pericarditis in uremia is usually considered to be a response to a chemical irritant, it is of interest to note that in 4 cases studied bacteriologically, streptococci were isolated from the pericardial exudate in each instance.

Arteriosclerosis of the superficial coronary arteries varied greatly in degree but tended to be only moderate. Frequently the vessels were

* Received for publication, November 14, 1941.

prone to appear in patients with hypertension. We were unable to confirm these observations. In our series there was no tendency for the degeneration to occur in any specific region. It was found in all layers, was usually diffuse and not limited to any particular zone. No relation could be found between its presence and the type of renal pathology or the blood pressure. There was slight fatty degeneration in 10 hearts, mild in 25, moderate in 13 and marked in 1.

Miliary myocardial necroses [3] were next in order of frequency. They were present in 35 instances, rare or scanty in 24, in moderate numbers in 10 and very numerous in 1. They seldom occurred as isolated lesions but were usually associated with acute interstitial myocarditis. This association suggests that these two lesions probably have a common underlying etiology.

Acute interstitial myocarditis was present almost as frequently as the myocardial necroses. It was found in 31 hearts, slight or mild in degree in 14, moderately extensive in 14 and very marked in 3. Bacteria were found in 3.

Acute miliary infarctions [4] were less common. In 10 hearts they were scanty, in 3 in moderate numbers and in 3 very numerous. In 2 hearts bacteria were present.

There were other lesions affecting the hearts in small number. There were 5 cases of rheumatic heart disease with valvular deformities. One was associated with extensive acute rheumatic myocarditis. Three hearts gave evidence of syphilis, twice affecting the aortic valve, once limited to the aorta. An acute vegetative endocarditis of the aortic valve was found twice. Two hearts had recent mural thrombi of the chambers; hemolytic streptococci were isolated from both.

Certain relations existed between the acute myocardal lesions and the renal pathology. Seven of the 8 cases of renal necrotizing arteriolitis had extensive severe damage of all types, although the miliary infarctions were the most prominent. Since these hearts had acute arteriolar changes, the presence of the myocardial damage could reasonably be attributed to them. About half of the nephrosclerotic patients had cardiac lesions of one type or another, miliary infarction being slightly predominant. Among those with pyelonephritis, the types of lesions exhibited a different proportion. Two-thirds, or 12, had acute interstitial myocarditis; one-third, or 6, had miliary necroses. Miliary infarctions occurred in only 3, 2 of whom had severe arteriosclerosis. Cardiac lesions among those with glomerulonephritis were uncommon.

Cardiac symptoms were present with the signs of uremia throughout the period of observation in 26 patients: congestive failure in 19 and coronary or left ventricular failure in 7. In the group with congestive

normal or slightly affected. Twelve had severe involvement: 4 each with pyelonephritis and nephrosclerosis, 3 with chronic glomerulonephritis and 1 with necrotizing arteriolitis. There was no close relation between the degree of coronary arteriosclerosis and either the renal pathology or the weights of the hearts.

The myocardium in the majority of the hearts was pale, mottled yellow or gray, sometimes with yellow or reddish brown streaking, and was of poor consistence. There were 2 instances of fresh massive cardiac infarction: 1 showed recent thrombosis and severe coronary arteriosclerosis; the other, normal coronary arteries without demonstrable

TABLE I

Weight of Heart According to Renal Pathology

	Weight of heart in gm.				
	450 or less	451–550	551–650	651 or more	Total
Pyelonephritis	10	1	2	5	18
Nephrosclerosis	3	3	1	3	10
Necrotizing arteriolitis	0	0	7	1	8
Acute diffuse glomerulitis	2	0	1	1	4
Chronic diffuse glomerulitis	7	1	1	0	9

thrombosis either grossly or microscopically. Healed massive infarctions were present in 3, all associated with the more severe degrees of arteriosclerosis of the superficial branches. One of these hearts had an interventricular aneurysm through which a small, healed, pin-point perforation connected the ventricular cavities.

The most interesting microscopical observation was the change found in the myocardial branches of the coronary arteries in the cases of renal necrotizing arteriolitis. The arterioles in 7 instances had an extreme endothelial hyperplasia, causing partial or complete occlusion. In 1 there was necrosis of the wall, polymorphonuclear infiltration and demonstrable bacteria. It apparently was a true infectious arteritis. A mild endothelial hyperplasia was found in 1 case of pyelonephritis. Moderate or marked atherosclerosis of the larger intrinsic arteries, with focal distribution, was present in 20 hearts, usually in parallel with similar changes of the superficial branches. Marked reduplication of the internal elastica was noted in 1 instance. No changes of any significance were found in 30 hearts.

The microscopical lesions of the myocardial fibers were of many types. Fatty degeneration was the most common, being present in 49 of the 50 hearts. Gouley [2] stressed the occurrence of a very fine, fatty degeneration of the myocardium showing a marked tendency to be most intense in the subepicardial layer. He stated that it was more

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ST. LOUIS

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failure, the renal changes were pyelonephritis in 6, nephrosclerosis in 6, necrotizing arteriolitis in 4 and glomerulitis in 3. In the coronary group, there were 2 each of pyelonephritis, nephrosclerosis and glomerulonephritis and 1 of necrotizing arteriolitis. In 25 of the 26 cases, acute myocardial lesions were present, exclusive of fatty degeneration. One case of pyelonephritis did not have demonstrable lesions.

Twenty-four cases were free of cardiac symptoms, except for terminal phenomena in a few instances. The renal lesions were pyelonephritis in 10, nephrosclerosis in 3, necrotizing arteriolitis in 3 and glomerulonephritis in 8. One case of arteriolitis had a terminal left ventricular failure of 7 hours' duration; the acute myocardial lesions were extensive and localized to the left ventricle and interventricular wall. The second case died suddenly; the lesions were very acute and concentrated on the left side. In the remaining cases, the cardiac lesions were either entirely absent or very moderate in extent.

Summary and Conclusions

The pathological findings in 50 hearts of patients dying in uremia are reported. No lesion was found which could be considered characteristic of the uremic state. An unusual endothelial hyperplasia of the cardiac arterioles was present in 7 of 8 cases with acute necrotizing arteriolitis of the kidneys. In no other type of renal pathology was there any correlation with the cardiac changes. A definite relation existed between the presence of acute lesions of the myocardial fibers and the occurrence of clinical signs of cardiac dysfunction.

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THE AMERICAN ASSOCIATION OF PATHOLOGISTS AND BACTERIOLOGISTS

Forty-Second Annual Meeting, Washington University School of Medicine, St. Louis, Missouri

April Second and Third, 1942

PRESIDENT HAYTHORN IN THE CHAIR

BUSINESS MEETING

April Second, 1942

Upon nomination of the Council, the following officers were elected by the Association:

President	PAUL R. CANNON
Vice-President	WILEY D. FORBUS
Secretary	HOWARD T. KARSNER
Treasurer	ALAN R. MORITZ
Incoming Member of Council	SHIELDS WARREN
Assistant Secretary	FRANCIS BAYLESS
Assistant Treasurer	GRANVILLE A. BENNETT

The Secretary announced the election of the following new members:

Arthur Charles Allen
W. A. D. Anderson
Alfred Angrist
Chapman H. Binford
Frank Bloom
Paul Edmund Boyle
Alfred Cohn
William Dock
John H. Fisher
Frank W. Foote, Jr.
Bruno Gerstl
Hugh R. Gilmore, Jr.
Leonard J. Goss
Sylvester Emanuel Gould
Thomas R. Hamilton
Frank Lappin Horsfall, Jr.
Thomas DeArman Kinney
Karl Lowenthal
Theodore L. Perrin
Abou D. Pollack
Geoffrey William Rake
Clara Raven
Cesare Tedeschi
Henry James Tweddell
George A. Walker
Tobias Weinberg
Robert J. Williams

The Secretary reported the reinstatement to membership of:

Charles B. McGlumphy
Margaret Warwick

SCIENTIFIC PROCEEDINGS

FATAL MATERNAL PULMONARY EMBOLISM BY AMNIOTIC FLUID. C. C. Lushbaugh (by invitation) and Paul E. Steiner, Chicago, Ill.

Abstract. On the basis of a study of ten women, together with experiments on animals, a new obstetric disease was recognized which has its distinctive symptomatology, etiology and pathology. The series was composed of cases which previously had been considered obstetric shock, idiopathic postpartum uterine atony with hemorrhage, acute pulmonary edema of pregnancy and as other obscure diseases, for all of which a causation had not previously been shown. Clinically the disease was characterized by shock coming on during labor or soon after its conclusion. Predisposing factors were uterine tetany or exceptionally strong uterine contractions, meconium in the amniotic fluid, intra-uterine death of the fetus, an oversize baby, multiparity and advanced age of the mother. The essential pathologic basis was found on microscopic examination of the lungs. It consisted of widespread embolism of small pulmonary arteries, arterioles and capillaries by the particulate matter found in amniotic fluid and by meconium. The disease was duplicated clinically and pathologically in rabbits and dogs by the intravenous injection of human amniotic fluid and meconium. The incidence of fatal cases was 0.2 per cent in general autopsies and 1 to 8,000 confinements. The incidence of sublethal cases is unknown, but these cases probably outnumber the fatalities. This disease was found to be the commonest cause of obstetric death during labor or in the first 10 hours of the puerperium.

AGING IN THE HUMAN PLACENTA. Louis M. Hellman and (by invitation) L. M. Flexner and A. Gellhorn, Baltimore, Md.

Abstract. The human placenta has a limited life span of approximately 280 days. During this brief period the processes of aging, normally occupying years, take place with great rapidity. Included under this heading is a great decrease in size and an increase in number of villi. The stroma becomes more compact and the fetal vessels increase in number. Degenerative changes occur in the covering coats, including the deposition of fibrin, fat and calcium. Inasmuch as the syncytium not only covers the villus but also lines the intervillous space, degenerative changes here are somewhat analogous to arterial changes associated with senility, and, similarly, thrombosis may ensue. On the positive side these old-age changes in the placenta result in greater ease of transfer from mother to fetus. This is demonstrated experimentally by a sixfold increase in the passage of radioactive sodium per gram of placenta from early pregnancy to term.

Discussion

(Dr. Carl V. Weller, Ann Arbor, Mich.) I would like to ask Dr. Hellman how sharp an end-point as to placental age can be established by the disappearance of the Langhans cells.

(Dr. Hellman) I do not think we know exactly—somewhere between the third and fourth month most of the Langhans cells disappear. Wislocki has said recently that if you examine the full-term placenta closely you can find Langhans cells.

FIBROUS PLEURAL ADHESIONS. Edward Smith (by invitation), St. Louis, Mo.

Abstract. Fibrous pleural adhesions were found in 66 per cent of 400 unselected autopsies upon patients varying from 1 to 89 years of age. The cases were studied grossly and microscopically without the aid of x-ray. The incidence of fibrous pleural adhesions increased from none at birth to 79 per cent in individuals 50

The Secretary announced the election of Esmond R. Long as representative of the Association in the Division of Medical Sciences of the National Research Council.

The Secretary announced the election of Jacob Furth to succeed Herbert Fox as a member of the Advisory Committee of the Lymphatic Tumor Registry.

The Secretary announced the election of Malcolm H. Soule to succeed himself in the Editorial Board of the *American Journal of Pathology.*

The Secretary announced that the next meeting of the Association will be held as the guests of the University of Chicago in Chicago, Illinois, April 1 and 2, 1943.

The Secretary announced the selection of James Ewing of New York as recipient of the Gold Headed Cane of the American Association of Pathologists and Bacteriologists.

The Secretary drew attention to the fact that at the last meeting of the Association the Council had recommended that in Article 2 of the By-Laws, the sentence "The Council shall consist of seven members elected by the Association, and the Secretary and Treasurer, ex officio," be changed to read, "The Council shall consist of seven members elected by the Association, and the Secretary, Treasurer and Editor of the *American Journal of Pathology* ex officio."

Upon motion, duly made and seconded, the above change in the By-Laws was approved by the Association.

Case 1. A white male, 80 years of age, complained of a mass in the neck enlarging for 5 years, recently causing hoarseness. A stationary mass in the same position in the neck had been noted for 30 years. The basal metabolic rate was plus 22. After operation there was rapid recurrence of the mass. At autopsy there were metastases to the lungs and mediastinum. The mass measured 20 by 15 by 8 cm. It was encapsulated and composed of a multilobular, firm, gray-white tissue. It showed atypical spindle cells arranged in broad bands and whorls. No characteristics of epithelial cells were found. In the pulmonary metastases several areas in suggestive acinar arrangement were shown to be vascular channels.

Case 2. A man, 69 years old, complained of a lump in the neck, difficulty in swallowing and swelling of the neck of 4 months' duration. The mass was in the region of the thyroid gland, was larger on the left and moved with deglutition. The basal metabolic rate was minus 19. The gross specimen was hard, encapsulated, multilobular, gray-white and weighed 352 gm. The microscopic picture varied. Large areas were composed of dense, poorly cellular fibrous tissue, with atrophic acini, and a diffuse lymphocytic infiltrate, often in follicles with prominent germinal centers. Scattered giant cells and areas of squamous metaplasia were present. The characteristics were those of struma lymphomatosa. The neoplastic areas were composed of broad sheets of atypical cells connected by fine processes. Reticulum was prominent and showed condensation in many areas. No characteristics of epithelial cells were found.

Discussion

(Dr. James Ewing, New York, N.Y.) The pictures of this case are quite characteristic of those which have appeared in the literature as sarcoma of the thyroid, of which the majority have been shown, on careful studies, to have definite epithelial structures, and that has been my experience with this characteristic structure—if you look long enough you will find undoubted epithelial areas. However, the authors seem to have been persistent and industrious in excluding such structures. They say they have cut many sections. Then the situation is this: Here is one case in which no epithelial structures are found, which therefore appears to be a sarcoma; but when this one case, or others like it, are observed in which no epithelial structures are found, and which are identical with those in which epithelial structures are found, is it not the conclusion that we have a real case of spindle-celled epithelial tumor of the thyroid?

As for the second case, it seems to me this is a rather characteristic type of small-celled carcinoma of the thyroid, which everyone knows is one of the great puzzles in diagnosis.

DIFFUSE MENINGIOSARCOMA OF THE BRAIN AND SPINAL CORD. S. R. Haythorn and (by invitation) William Shapera and H. C. Stewart, Pittsburgh, Pa.

Abstract. Two cases of fibroblastic meningioma, both of which presented histologic evidences of malignancy, were reported. In the first case the tumor involved the basal meninges and the spinal meninges from the region of the infundibulum to the filum terminale. It ensheathed the entire spinal cord like a stocking and broke into fibrillary branches which surrounded the sheaths of the nerves composing the cauda equina. It apparently arose from the pia and arachnoid, caused distortion and atrophy of the cord, followed the meningeal folds into the fourth ventricle, surrounded the chorioid plexus and formed a small metastasis by extension into a part of the posterior lobe of the pituitary gland.

In the second case the diagnosis was made at autopsy. A hemorrhage the size of a man's fist was found occupying the left supra-orbital space. The hemorrhage was free from the pia but firmly attached to the dura. The region of the second

years of age. Lungs with calcified nodules and apical scars did not show a significantly increased incidence of fibrous pleural adhesions. Of 49 cases with a past history of pneumonia and/or pleurisy, 94 per cent showed adhesions. The cases with a past history of pneumonia and/or pleurisy showed an average area of pleural involvement twice that of the average of comparable age groups without histories of pneumonia and/or pleurisy. Isolated adhesions were more common than diffuse adhesions except in the pneumonia group. The most common location for adhesions was over the lower lobes posteriorly. Microscopically, the great majority of fibrous pleural adhesions were associated with slight fibrous thickening external to the subpleural elastic layer. There was usually no change in the underlying pulmonary tissue. Several other frequently occurring clinical and pathological entities were found to have no apparent relationship to fibrous pleural adhesions.

AMYLOID GOITER. George A. Walker (by invitation), Kansas City, Kans.

Abstract. Fifty-six cases in which amyloid could be demonstrated in the thyroid gland have been reported in the literature. Of these, 35 showed enlargement—true "amyloid goiter." Amyloid deposits occurred as a part of the secondary or generalized type of amyloidosis in 40 cases. The commonest associated primary disease is pulmonary tuberculosis; purulent bronchitis with bronchiectasis or neoplastic processes are less commonly the precursors. Two cases of amyloid thyroid without amyloid in any other organ have been reported (primary amyloidosis of the thyroid).

This paper reported two cases of amyloid in the thyroid gland. The first patient was a white male, 60 years old, who had severe dyspnea due to marked enlargement of the thyroid beginning 3 years before admission to the hospital. At autopsy, tuberculous bronchopneumonia with cavitation and generalized amyloidosis with involvement of the thyroid were found. The thyroid weighed 280 gm., contained very few follicles and showed marked lipomatosis in addition to extensive deposits of amyloid. The second patient was a boy, 16 years old, who developed generalized amyloidosis while under treatment for the active tuberculosis which caused his death. At autopsy, in addition to pulmonary tuberculosis, there was generalized amyloidosis. The thyroid gland weighed 10 gm. and consisted almost entirely of amyloid.

In no reported case has there been any evidence of thyroid deficiency.

Discussion

(Dr. Jack D. Kirshbaum, Chicago, Ill.) Were either of these two cases associated with a severe anemia?

(Dr. Walker) No, sir; none of the cases reported have been associated with anemia, although the clinical and laboratory findings in every case were not available. Another interesting fact is that in spite of extensive replacement of the thyroid parenchyma by amyloid, in no case has there been any evidence of thyroid insufficiency. One of our cases showed an elevated basal metabolic rate, but this was probably due to dyspnea.

PRIMARY SARCOMA OF THYROID GLAND. TWO CASES. Mendel Jacobi and Herman Bolker, Brooklyn, N. Y.

Abstract. Two cases were presented which showed no carcinomatous elements and which clearly arose from the thyroid gland. One of these was of further interest because it also presented the histologic picture of thyroiditis of the struma lymphomatosa type. There is no reported case of this disease with malignant change.

H. E. Robertson some years ago, in which he reported the examination of 16 polyps removed at autopsy from one individual and found only one of them to be malignant. Unless frank malignancy is found in the section, the surgeon should share the responsibility of a negative diagnosis with the pathologist.

Dr. Bell, do you have anything to say about this?

(Dr. Bell) The same difficulty is encountered by us, and if we do not get the base of the polyp we take no responsibility for the diagnosis. The surgeon must produce the base of the polyp or take the responsibility himself. If the polyp is on a narrow pedicle, it can usually be regarded as benign. Many times we get the tip of the polyp, and we cannot tell whether it is malignant. I think it is dangerous to call polypi malignant merely because we see malignant changes on the surface, since a large percentage of these show no invasion of the base.

(Dr. N. Chandler Foot, New York, N.Y.) I think a very important thing in this connection is for the surgeon, when he sends the biopsy, to send a description of the tumor from which he took it. We seldom get the entire tumor at biopsy. It was only a month or two ago that I saw an instance of this sort, when a small piece of malignant polyp was excised and examined by a local pathologist, and without any knowledge of the gross appearance the diagnosis of carcinoma was made. The surgeon did a combined abdomino-perineal resection of the rectum, and when the specimen came through there was nothing but a so-called adenoma malignum on a long pedicle. The surgeon was very much disturbed when we failed to make the diagnosis of carcinoma of the rectum. The whole thing could have been avoided had the surgeon told the first pathologist where he took his biopsy specimen and the appearance of the tumor. As Dr. Bell says, the condition of the pedicle of these tumors is the most important point of consideration.

(Dr. Helwig) I did not mean to imply or give the impression that these polyps with foci of carcinoma should be regarded as obvious or manifest carcinoma, and the colon resected. I wish to emphasize what Dr. Foot has told you, that if a small portion of the polyp—for instance, the tip—is removed and sent to the pathologist, he cannot do otherwise than make a diagnosis of carcinoma. He cannot understand the structure of the entire polyp from such a biopsy. On the other hand, the pathologist commits a grave error if he does not insist that the entire polyp be removed.

MASTOPATHIA CYSTICA AND MAMMARY CARCINOMA. Carl V. Weller and (by invitation) James W. Logie, Ann Arbor, Mich.

Abstract. The relationship of cystic disease of the breast to mammary carcinoma has long been a subject of controversy, yet practical observation frequently reveals the concomitance of the two conditions. The criteria for the recognition of mastopathia cystica, as applied to the present study, included dilatation of ducts with accompanying fibrosis; areas of "pale-celled" hypertrophy of ductal epithelium, of the type found in the apocrine sweat glands; papillary ingrowths, to varying degree, into cystic ducts, and, frequently, hyperplasia of terminal ducts and acini. Operative material from the breasts of 330 women was reëxamined. Of these specimens, 118 were carcinomatous, and of these 67 showed coexisting mastopathia cystica. Of 212 breasts without carcinoma, 82 showed mastopathia cystica. Construction of a fourfold table and application of the X^2 test to these data indicated that the chance of obtaining such a degree of concomitance, if mastopathia cystica and mammary carcinoma are independent, is less than 1 in 1000. In practical diagnostic experience, carcinoma is frequently found arising in areas of mastopathia cystica. This was true of 13.5 per cent of the cancers of the breast included in this analysis. Upon both statistical and histopathologic grounds a causal relationship between mastopathia cystica and mammary carcinoma must be accepted.

and third frontal gyri showed pressure distortion from the hemorrhage and an area of softening. On section, a thrombosed arteriole was found in the apex of the wedge-shaped, softened area. This apparently marked the source of the hemorrhage. On microscopic examination, the arachnoid was found to be the seat of a tumor which was exactly the same in structure as that found in the first case. The tumor tissue was attached to the dura but separated from the cortex by the pia. The tumor was diffuse in the sense that it was not encapsulated and spread along the arachnoid zone.

Special stains confirmed the fibroblastic nature of both tumors.

THE RELATION OF POLYPS TO CARCINOMA OF THE COLON. Elson B. Helwig (by invitation), St. Louis, Mo.

Abstract. The colons from 1,358 consecutive autopsies were examined for the presence of mucosal polyps. All other lesions, including both tumors of other types and lesions obviously a result of infection, were excluded from this study. Of the 1,358 colons examined, 128, or 9.4 per cent, contained polyps. Of these 128 cases, 121 were available for detailed microscopic study. After 1 case of familial polyposis was excluded, the remaining 120 cases contained a total of 237 polyps. The 1,358 patients were grouped according to age and the percentage of patients with mucosal polyps of the colon in each age group was determined. The incidence of polyps increased from 3.7 per cent in the first decade to 23 per cent in the eighth decade.

Each polyp was analyzed histologically by step or serial sections for the presence of carcinoma. In determining the presence of carcinoma in a polyp the general criteria suggested by Swinton and Warren were used. These were the presence of two of the following three criteria: (1) anaplasia, (2) irregularity of architecture and (3) invasion. Eleven of the 120 examples of polyps of the large intestine showed foci of carcinoma within a polyp. The incidence of polyps with foci of carcinoma was greatest in the seventh decade. Eight of the 11 cases of polyps with foci of carcinoma were situated in the rectum or sigmoid colon. There were 22 cases of manifest carcinoma of the large intestine among the 1,358 intestines examined. The incidence of manifest carcinoma of the colon was greatest in the seventh decade. Fifteen of the 22 cases of manifest carcinoma occurred in the rectum or sigmoid colon.

Discussion

(Dr. Emmerich von Haam, Columbus, O.) Was the last picture shown one of the cases that died of carcinoma, or was it one of the incidental findings?

(Dr. Helwig) The polyp in the last case was an incidental finding. The first polyp that was shown came from a case in which there was also an obvious or manifest carcinoma.

(Dr. E. T. Bell, Minneapolis, Minn.) I think it might be brought out that while we recognize these as malignant changes in polypi, in talking to the surgeon about it we should not advise a radical operation unless there is invasion of the base, and we should have some kind of name to convey that idea to the surgeon. If one calls this a carcinoma, the surgeon may do a radical operation, which I do not think is necessary when the malignant changes are found only at the surface of the polyp.

(Dr. S. R. Haythorn, Pittsburgh, Pa.) Pathologists are often asked to look at polyps and decide the question of malignancy for the surgeon. Usually such biopsies do not include the base, so that it is impossible to tell whether invasion is present or not. Where the change is early and limited to the superficial portion of the polyp, the decision is a difficult one to make. I was impressed by a paper by Dr.

(Dr. Weller) That assumption is not made for a single one. We are considering coincidence or lack of coincidence.

(Dr. Hansmann) The fact is that at that age there might be a coincidence that might be accidental.

(Dr. Weller) That is what we are trying to investigate: whether it might be casual or causal.

THE CYTOLOGIC CHANGES IN CARCINOMA OF THE HUMAN PROSTATE GLAND FOLLOWING ADMINISTRATION OF DIETHYLSTILBESTROL AND DIETHYLSTILBESTROL DIPROPRIONATE. John R. Schenken and Edward L. Burns, New Orleans, La.

Abstract. The effects of diethylstilbestrol diproprionate on carcinoma of the prostate gland were studied in six patients. In five cases biopsies on the same patient were made before and after treatment. In one case studies were made only on tissue removed after treatment. The patients received from 1,100,000 to 1,600,000 international units over periods varying from 23 to 65 days. Control observations were made not only on the 5 neoplastic tissues removed before hormone therapy was started, but also on the tissues of 44 cases of untreated carcinoma of the prostate gland. As compared to the controls, microscopic studies showed, without exception, that treatment was associated with retrogressive tissue changes in the neoplastic cells. In one case identical changes were noted in the metastatic tumor cells of an inguinal lymph node. In all cases the changes consisted of extensive vacuolization of the cytoplasm and pyknosis or karyolysis of the nucleus. In some cases these changes resulted in complete resorption of the cell, leaving clear spaces in the stroma of the prostate gland.

Discussion

(Dr. Emmerich von Haam, Columbus, O.) I should like to ask if the patients showed nausea or any of the other described phenomena following diethylstilbestrol diproprionate, and I should also like to know if the drug was given by intravenous injection, or by what route.

(Dr. Michael B. Shimkin, Baltimore, Md.) I have three questions: Has this form of therapy been used for other malignant neoplasms, particularly those with metastases to bone; second, have the authors any studies to show what changes take place in bony metastases histologically; and third, is there any information available as to what happens in the testes in these cases with particular reference to the interstitial cells?

(Dr. Howard T. Karsner, Cleveland, O.) Has it been possible to check on the output of acid phosphatase?

(Dr. Schenken) In reply to Dr. von Haam's question, there have been some side effects; some have been nauseated and some have vomited, although we have been able to take care of this rather easily by reducing the dosage for a short period of time, after which the dosage is then resumed. The drug was administered intramuscularly.

In reply to Dr. Shimkin, we are trying it on other malignant diseases in which the roentgenologist and the surgeon both have given us a written statement that they have given up. We are not trying it on cases other than those. So far we have nothing definite to report, although there are a few cases in which there may be some encouraging signs.

We have had no bone biopsies as yet, so we do not know anything about the histological changes, but this one patient was studied very thoroughly by the roentgenologist, who reported restoration of the normal bony structure in some areas. No testes have been removed following this treatment, as yet.

Dr. Karsner, we have not determined the acid phosphatase, but such studies are being done now.

Discussion

(Dr. Francis Carter Wood, New York, N. Y.) It seems to me this is statistically a perfectly sound paper. Certainly, mastopathia cystica is the expression of a reaction to a chronic irritative agent. Also experimental work with animals shows that anything which stimulates proliferation of glandular tissue may induce cancer. Claims have been made that by the injection of theelin, for example, a large number of carcinomas of the breast can be produced in animals. The animal used in many cases was the mouse, certain strains of which are very susceptible to such stimulation. The statement has also been made that a large number of carcinomas may be produced by theelin in the rat, but the growths are the so-called "microscopic carcinomas," and when the animals were kept for a year, no carcinomas developed, except in a very small percentage. Nevertheless, any irritative and reparative process like that seen in the human breast in mastopathia cystica which induces proliferation of epithelium must be considered potentially dangerous.

(Dr. James Ewing, New York, N.Y.) Can Dr. Weller point out any feature of cystic mastitis which is likely to become cancerous? Furthermore, I should like to ask if any conclusion was drawn as to the relation between stagnation of secretion and the origin of carcinoma.

(Dr. Weller) To answer Dr. Ewing as best I can, in the first place, in general we find that the papillary ingrowths into large cysts are seldom the apparent origin for carcinoma. More often the more solid proliferations in the terminal ducts or dilated acini are the apparent sources of the carcinoma, and frequently the only difference between a diagnosis of mastopathia cystica and one of carcinoma depended upon demonstrating infiltrative growth.

I think there was some local relation to stagnation. The retention of material in small cysts may be of some consequence in determining the local development of carcinoma. I intentionally kept away from such considerations in this presentation, for they would require a large group of lantern slides to illustrate the relations.

(Dr. E. T. Bell, Minneapolis, Minn.) I would like to ask if Dr. Weller has ever followed a group of women with cystic mastitis where the breast was not removed, and if so, what were the results? I have followed such a group of around 50 cases for 5 to 10 years and have not seen a carcinoma develop. If we follow Dr. Weller's recommendation, the breast should be amputated for cystic disease. Some of these women are in their twenties. Does Dr. Weller recommend complete mastectomy in young women with cystic disease?

(Dr. Weller) I did not say that women with cystic disease of the breast should have amputation of one or both breasts. I did say such a woman was a candidate for close watching, for frequent clinical examination and check-up. We have not followed a large group such as Dr. Bell inquires about, and I am well aware of the fact that, according to the literature, some follow-ups show a very small proportion of carcinoma after a 5-year period.

Dr. Robert C. Grauer, Pittsburgh, Pa.) Does not Dr. Weller think that the important point in determining whether or not amputation of the breast should be recommended is the age incidence? Because of the importance of ovarian activity in stimulating acinar proliferation, where the younger age group is concerned, there is more chance for malignancy to supervene in these patients than in those of age 50 or more.

(Dr. Weller) That might well be true, but I am still not recommending amputation of the breast for mastopathia cystica, regardless of the age.

(Dr. George H. Hansmann, Milwaukee, Wis.) I do not know much about the formula shown, but I should like to ask if it assumed that all carcinomas when present have as their etiological factor chronic cystic mastitis.

renal arteriolosclerosis increases in frequency as the blood pressure increases, but is found in about one-half of those with systolic pressures below 140 mm. Hg. It may be stated, therefore, that in diabetes, renal arteriolosclerosis may occur independently of hypertension and cardiac hypertrophy.

Intercapillary lesions of different degrees of intensity were found in 20.5 per cent of diabetic patients—15.8 per cent of the males and 26.9 per cent of the females. About one-third of the intercapillary lesions were of spherical shape and the remainder were diffuse. The hyaline masses between the capillaries are formed chiefly from thickening and splitting of the capillary basement membranes. There is always an associated severe hyalinization of the afferent and efferent glomerular arterioles. In chronic glomerulonephritis there is commonly a central hyalinization of the glomerular lobules which, however, may usually be distinguished from the diabetic lesion.

There are no definite clinical features by which diabetes with intercapillary lesions can be distinguished from diabetes without glomerular alterations.

Discussion

(Dr. D. Murray Angevine, Wilmington, Del.) If the lesion is in the wall of the capillary, I should like to ask Dr. Bell why he calls it intercapillary rather than intracapillary.

(Dr. Howard C. Hopps, Chicago, Ill.) I should like to know if there were any changes in the juxtaglomerular apparatus associated with these changes in the arterioles.

(Dr. Grawitz, New York, N.Y.) I should like to ask if there is any correlation between the changes seen histologically and the renal function.

(Dr. Bell) In regard to Dr. Angevine's question, I used the term "intercapillary" because everyone else uses it; the lesion is conspicuously in the center of the lobule and looks as if it were between the capillaries. I do not like that name. I want to emphasize that we may find practically the same lesion in glomerulonephritis and that it consists of a thickening of the capillary walls rather than of a deposit between them. I do not think intercapillary is a satisfactory term.

There are no changes in the juxtaglomerular apparatus.

As to the correlation with renal function, some of the extreme lesions show a depression of renal function. When the changes are very pronounced in all the glomeruli, the patient will have a nitrogen retention. The blood pressure is in general higher with this lesion, and there is also a severe involvement of the afferent arterioles. It never occurs except with a severe involvement of the afferent as well as the efferent arterioles.

STUDIES ON HODGKIN'S DISEASE AND ITS RELATION TO INFECTION BY BRUCELLA.
Wiley D. Forbus and (by invitation) D. W. Goddard, George Margolis, Ivan W. Brown, Jr., and Grace P. Kerby, Durham, N.C.

Abstract. The investigations reported in this paper deal with the problem of a possible etiological relationship between *Brucella* and Hodgkin's disease as suggested by previously published observations from our laboratories. Extended studies on the incidence of antemortem recovery of *Brucella* from 24 typical cases of Hodgkin's disease yielded the following results: *Brucella* was recovered from 9 of 17 cases by blood culture and from 9 of 23 cases by culture of excised lymph nodes; 4 of 23 cases yielded *Brucella* by both blood and node culture and 14 of 24 cases yielded *Brucella* by either blood or node culture. Coincident with the studies on cases of Hodgkin's disease a series of 84 cases of lymphadenopathy, not Hodgkin's disease, was studied by the same cultural methods. This series included a variety of pathological processes. Seven of the 84 cases yielded *Brucella*

THE RATE AND PERIODICITY OF MITOTIC ACTIVITY IN EXPERIMENTAL SQUAMOUS CELL CARCINOMA OF MICE. Charles M. Blumenfeld, Cleveland, O.

Abstract. The growth of tumors is independent; of normal tissues, dependent. In both, growth by increase in the number of cells is accomplished largely by mitosis. Rate and periodicity of mitosis are ultimate expressions of factors regulating it. Therefore a comparison of rate and periodicity of mitosis in a tumor and in the normal tissue from which it arises may shed light on certain fundamental characteristics of the tumor cell. Squamous cell carcinoma of skin was produced by painting the interscapular region of 2-month-old CBA mice with a 0.3 per cent solution of methylcholanthrene in benzene. Malignant tumors and normal skin have been obtained from 36 animals killed at 8 a.m., 12 m., 4 p.m., 8 p.m., 12 p.m. and 4 a.m. Mitotic activity in each specimen was determined by counting the mitoses in 500 fields. All data were subjected to statistical analysis. Mitosis in normal epidermis showed diurnal rhythm, with 247 mitoses per 500 fields at 12 m., and 60, at 12 p.m. The difference is significant. In the tumors, mitotic activity showed no rhythm, remaining, during the 24-hour period, at a level not significantly different from maximum normal activity, *viz.*, 206 mitoses per 500 fields. The following hypothetical explanation is offered: during the process of differentiation and organization to become part of an organ, normal cells acquire a component which, responsive to the stimulus causing function of the organ of which they are a part, shifts them between vegetative and functional activity. Periodic rise in function of an organ causes periodic fall in vegetative activity; therefore, rhythmicity of mitosis. In tumor cells the loss or diminution of this component results in no, or lessened, diversion of cells from vegetative to functional activity. Consequently, the tumor exhibits no periodic depression of its mitotic activity.

THE INCIDENCE OF ALTERATIVE GLOMERULITIS (KIMMELSTIEL) IN ELDERLY PATIENTS. William A. Morningstar (by invitation) and Paul Gross, Pittsburgh, Pa.

Abstract. The patients selected for study were those of 60 years or older who had no clinical or pathologic evidence of glomerulonephritis or significant involvement from pyelonephritis. Of 174 cases investigated, 50 per cent showed no significant glomerular change. There were 30 cases (17 per cent) in which one-half or more of the glomeruli in the slides available for study showed alterative glomerulitis. Cardiac hypertrophy (375 to 540 gm.) was present in 16 of these, while in 11 the cardiac weights ranged between 225 and 350 gm. Although blood chemical studies had not been done on many of these patients, when available the values were within normal limits. From the clinical point of view, none of these patients was uremic.

RENAL LESIONS IN DIABETES MELLITUS. E. T. Bell, Minneapolis, Minn.

Abstract. A microscopic study of the kidneys was made in 460 diabetic patients. Diabetes is about twice as frequent in females as in males. Hypertension (systolic pressure of 150 mm. Hg or higher) was present in 29.3 per cent of those below 50 years of age and in 65.6 per cent of those over 50 years old. Hypertension is definitely more frequent in diabetic than in non-diabetic patients of corresponding age.

Renal arteriolosclerosis was found in 31.8 per cent of those under 50 years of age and in 77.9 per cent of those over 50 years old. The arteriolar lesion is a definite subintimal deposit of a hyaline substance, usually present in a thick layer. Renal arteriolosclerosis is somewhat more frequent in those with large hearts but is present in two-thirds of those with a cardiac weight of 250 to 300 gm. Similarly,

degree of fibrosis. In the experimentally produced lesions of the testes, such as appeared in one of our hogs, there was not only fibrosis but also considerable calcification. The organism used to infect this animal was isolated from a scarred and calcified lesion in the spleen of a hog sent to us by Dr. Davis.

(Dr. Howard T. Karsner, Cleveland, O.) Will Dr. Forbus be good enough to recapitulate the results of cultural studies in other regions of the country?

(Dr. Forbus) I know of only one instance of recovery of this organism from a case of Hodgkin's disease in other laboratories than our own. This positive culture was accomplished by Dr. Jeter of Oklahoma City. The organism was grown from a lymph node. Sections of the lymph node and a culture of the organisms were sent to us, and we were able to confirm Dr. Jeter's observations. That patient is now dead, presumably from Hodgkin's disease, but no autopsy was performed. I have no information regarding cultures by anyone else. It should be emphasized that the cases we have studied are not all from our own locality. Of the fatal cases studied at autopsy, two were from distant parts of the country. One was from Cincinnati; another was from Florida—a young boy who made seasonal trips from Florida to Detroit. His home was in the latter place. We have recently studied a case for a period of 6 months—a boy from Washington, D.C.—who finally died of the disease. Unfortunately, we could not obtain permission for autopsy, but we did have lymph node tissues, and there was no question, either clinically or histologically, that the case was one of Hodgkin's disease. After prolonged effort we recovered the organism from the boy's blood. It took 35 cultures to do it.

(Dr. E. T. Bell, Minneapolis, Minn.) Is the milk pasteurized in the part of the country from which the cases came?

(Dr. Forbus) In certain sections, yes, and in others, no.

(Question from audience) I should like to ask if serological studies have been done to prove the presence of antibody.

(Dr. Forbus) Yes, all cases have been studied for the presence of antibodies, and the interesting and puzzling thing is that we have not found them present in any one of the Hodgkin's cases.

(Dr. R. D. Lillie, Bethesda, Md.) Does it not account for the more frequent presence of organisms in Hodgkin's nodes that no antibodies are formed by neoplastic reticulo-endothelial tissue?

(Dr. Forbus) That is a thesis that I would not like to attempt to develop, since we do not look upon the reticulo-endothelial proliferative reaction in Hodgkin's disease as neoplastic in the usual sense of the term. I have no explanation for the absence of antibodies, but I think it is correct to say that this finding is not wholly inconsistent with the presence and pathogenic activity of *Brucella* in the individual.

(Dr. Karsner) With or without Hodgkin's?

(Dr. Forbus) Either with or without.

(Question from audience) I did not hear whether Dr. Forbus said the strain of the organism was porcine or bovine.

(Dr. Forbus) We have recovered *Brucella abortus* only occasionally. The organisms are usually *Brucella melitensis* or *Brucella suis*.

(Dr. George H. Hansmann, Milwaukee, Wis.) Would inoculation of guinea pigs give a positive reaction?

(Dr. Forbus) In one series of studies we inoculated guinea pigs directly with Hodgkin's tissue obtained at autopsy but we never succeeded in producing lesions; but with the organisms that we obtained by cultures of the same tissues we did succeed. These animals were not studied extensively for antibody production but the organisms were consistently recovered from the lesions which were typical of brucellosis.

(Dr. Hansmann) What is the dosage of the organisms injected?

either by blood or node culture. Of the 24 cases of Hodgkin's disease, 8 are now unreported. 6 are living and 10 have died of Hodgkin's disease.

The above antemortem studies have been supplemented by studies on 14 fatal cases of Hodgkin's disease (including 6 of the above series), 12 of which were cultured during life and 9 of which were cultured at autopsy. Ten of the 12 cases cultured antemortem yielded *Brucella* by blood or lymph node culture and 6 of the 9 cases cultured at autopsy were positive for *Brucella*. Four of 7 cases yielded this organism both antemortem and at autopsy, and 12 of the 14 cases yielded *Brucella* either before death or at autopsy. None of the cases of Hodgkin's disease showed specific antibody reactions in what we regard as significant titer. In one series of 103 cases, not Hodgkin's disease, studied at autopsy, *Brucella* was recovered once; in a second series of 68 cases this organism was recovered from 10 cases, the combined figure being, therefore, 11 out of 171, or an incidence comparable to that found in the non-Hodgkin's cases studied antemortem.

The strains of *Brucella* obtained at autopsy have been employed in a study of experimental brucellosis in a variety of animals including guinea pigs, rabbits, dogs and hogs. These animals react to intravenous and intraperitoneal inoculations by the production of granulomatous lesions in the peritoneum, and especially in the lymph nodes. The lymph node lesions, particularly in the hog and the guinea pig, are characterized by extensive proliferation of the reticulo-endothelial cells, sometimes so pronounced as to replace almost completely the lymphoid structures. The reaction is sometimes followed by necrosis, and is always accompanied by outpouring of eosinophils and plasma cells. Multinucleated giant cells are typical of the fully developed lesions. In principle and in some of the details the reaction resembles that of Hodgkin's disease. The type of parasitism produced by *Brucella* in the experimental animal is peculiar in that the organisms seem to prefer, if they do not require, an intracellular position for their propagation. This may have important bearing upon the rapidity with which they leave the blood stream when injected intravenously in large numbers and also upon the difficulty of recovering them from animals known to be heavily infected.

Discussion

(Dr. Seaton Sailer, Cincinnati, O.) What effect does radiation have on the bacteria, and on the growth of the lesion?

(Dr. Forbus) We have had no experience with the effects of radiation on this organism.

(Dr. James Ewing, New York, N.Y.) I had the great pleasure and good fortune to have Dr. Forbus bring his material and to study it with him at considerable length, and I will say with a very sympathetic attitude on my part. It is perfectly obvious that he is producing a characteristic granuloma with this organism. After going over much material with him, I made the comment which I still hold: that it just misses duplicating the series of lesions which we see in Hodgkin's disease. I think the lesions on the whole were more acute and exudative. In the early stages a cellular reaction is very marked, but the late fibrosis and the essential characteristics of the Hodgkin's lesion were missing. As I remember it, Dr. Forbus was to gather observations on that and report later as to whether he had any chronic cases which did more thoroughly reproduce the lesions of Hodgkin's disease.

(Dr. Forbus) We have had no opportunity to study brucellosis in experimental animals for a longer period than about 400 days. However, that is a long time in the life of a hog—one of the animals that we employed. Ordinarily this animal lives about 6 months, or rather, it is killed at the end of 6 months, so we do not know what happens in the naturally acquired disease thereafter. Nevertheless, I think you would find it very interesting to look at Dr. Davis's demonstration of spontaneous brucellosis in hogs since one of his cases does show a considerable

during drowning due to the diffusion of electrolytes through the walls of the pulmonary capillaries. The nature of and the extent to which such changes may occur have not been observed under controlled conditions and the manner in which these changes differ from those which occur after death independently of drowning has not been investigated.

Changes in the concentration and distribution of chlorides and magnesium were studied in four groups of dogs. In the first, the animals were sacrificed by mechanically induced asphyxia (not drowning) and allowed to remain in the open air for as long as 72 hours after death. The rate of postmortem change in these animals was regulated by keeping their bodies at different temperatures. In the second group, the animals were sacrificed by mechanical asphyxia and were then submerged in either fresh or sea water throughout the period of postmortem observation. In the third group, the animals were drowned in fresh water, and in the fourth, they were drowned in sea water. Blood was withdrawn from the right and left sides of the heart at intervals after death and the chloride and magnesium content of both cells and plasma was observed.

In the control animals, it was found that diffusion of both chlorides and magnesium between cells and plasma began soon after death and that within 12 hours the normal antemortem differences in their concentration in cells and plasma had usually almost entirely disappeared. The chloride content of the heart's blood underwent progressive reduction during putrefaction, whereas the magnesium content became progressively higher. Submersion after death did not alter the rate or character of these changes.

In animals drowned in fresh water there was a sharp drop in the chloride content of the blood in both sides of the heart during the agonal period. The reduction was greater than that observed in any of the control animals. The reduction was more pronounced in the left than in the right side. The difference between the concentrations in the two sides of the heart diminished as putrefaction progressed. Marked hemolysis of left ventricular blood was observed within 15 minutes after death.

In animals drowned in sea water it was observed almost immediately after death that both the magnesium and the chlorides in the heart's blood were elevated, the former proportionately more than the latter. The elevation was more pronounced in the left than in the right side of the heart. Hemolysis of blood was delayed and the magnesium content of the stomach was abnormally high.

ALLERGIC REACTIONS TO SURGICAL CATGUT—AN EXPERIMENTAL STUDY. Howard C. Hopps (by invitation), Chicago, Ill.

Abstract. Hypersensitivity to catgut has been produced in rabbits as demonstrated by positive skin tests and by the presence of humoral antibodies. Catgut suture (powdered), sheep intestine and sheep serum are essentially similar in their ability to stimulate the production of antibodies specific for catgut. Heterophil antigen (guinea pig kidney) does not stimulate perceptible hypersensitivity to catgut; neither do individuals with high heterophil antibody titers (following or during the course of infectious mononucleosis) possess demonstrable antibodies for catgut.

Studies of tissue reaction to catgut suture in normal and hypersensitive animals were presented. These included observations on suture implants into the anterior chamber of the eye and histological studies of catgut suture in muscle and subcutaneous tissue. It was concluded that allergy to surgical catgut does not significantly influence early tissue reaction (12 hours to 3 days) to catgut suture.

(Dr. Forbus) Approximately 10 billion organisms for the dog and hog. In one case we used 30 billion organisms; with the guinea pig we used about 1 to 3 billion.

(Dr. Emmerich von Haam, Columbus, O.) Did you introduce a stock culture, or do you think the organism has to be isolated from these cases?

(Dr. Forbus) I see no reason why the lesions cannot be reproduced with a stock culture, because the experiments on the dog and hog were done with one of the strains previously used in our guinea pig experiments and that strain had been kept in the laboratory for 6 or 8 months.

(Dr. Israel Davidsohn, Chicago, Ill.) Did you notice the production of antibodies in the experimental animals?

(Dr. Forbus) The typical response occurred in the hog and the dog.

(Dr. Davidsohn) I may add in regard to the production of antibodies in Hodgkin's disease, that in a series of cases studied by me some of the normal antibodies, namely the iso-agglutinins and the agglutinins for sheep red cells, were present in about the same titer as in normal individuals.

ANTEMORTEM AND POSTMORTEM DIFFUSION OF ALCOHOL THROUGH THE BLADDER MUCOSA. Alan R. Moritz and Walter W. Jetter, Boston, Mass.

Abstract. In each of a series of dogs the bladder was isolated from the kidneys by ligation and section of the ureters. Subsequent to this procedure arbitrary initial differences were established between the concentration of alcohol in the blood and its concentration in the urine contained in the isolated bladder. All concentrations were within the normal range in the sense that none of them was higher than might result from drinking an alcoholic beverage. The initial disparity between the concentration of alcohol in the blood and that in the bladder urine was greater in some animals than would be likely to occur in non-experimental conditions. It was found that the bladder urine does not represent an unaltered pool of ureteral urine so far as its alcoholic concentration is concerned. Alcohol passes into the urine by way of the mucosa of the bladder when the initial alcohol concentration of the urine is disproportionately low, and out of the urine by the same route if its alcoholic concentration is disproportionately high. The passage of alcohol through the bladder mucosa may occur in either direction after death and the direction of its diffusion is determined by the disequilibrium in its relative concentration in blood and urine at the time of death. It is not necessary that either the absolute or the relative concentrations of alcohol in urine and blood be different from those which occur normally in order for an interchange of alcohol between bladder urine and blood to occur. In both living and dead animals the passage of alcohol through the bladder's mucosa tends to bring its concentrations in blood and urine closer to, rather than farther from, a state of equilibrium.

The fact that alcohol may pass in either direction through the mucosa of the bladder tends to enhance rather than to detract from the clinical and medicolegal significance of any disproportion which may exist between the alcoholic concentrations of simultaneously collected samples of blood and bladder urine.

CHANGES IN THE MAGNESIUM AND CHLORIDE CONTENT OF THE BLOOD FOLLOWING DROWNING IN FRESH AND SEA WATER. Walter W. Jetter and Alan R. Moritz, Boston, Mass.

Abstract. The pathological changes are frequently inconclusive in establishing the cause of death of persons whose bodies have been recovered from water. In such instances the opinion that death has been caused by drowning is likely to be based in part on the circumstances in which the body was found and in part on the fact that the postmortem examination failed to disclose any other cause of death. It has long been known that the chemical constitution of the blood may be altered

the disease was markedly altered, or recognizable signs of tuberculosis were missing at necropsy. That definite proof might be assembled which would indicate with reasonable certainty that the favorable results seen after treatment with promin had occurred in animals in which lesions of tuberculosis were demonstrably present before treatment was started, this study was made. A total of 51 guinea pigs were inoculated subcutaneously with tubercle bacilli, strain $H_{37}RV$ (dose 0.0005 mg. subcutaneously). Forty-two days later all were found to be sensitized to tuberculin and were divided into two groups. Group 1 consisted of 31 animals; these were not treated. Group 2 consisted of 20 animals; this was the treated group. Beginning with the 42nd day, they received promin (given orally in the food), 300 to 400 mg. per animal each 24 hours. One week after treatment began, biopsy specimens of the liver were obtained from the animals in the treated group and from 12 of the animals in the control group. The experiment was terminated when the last of the controls died. This was 224 days after infection. There were still living at this time 81 per cent of the treated animals. The biopsy material revealed definitely that tuberculosis was present in the livers of both treated and untreated animals at the time treatment was started. These findings were compared eventually with the findings obtained from a study of the same livers at the time of necropsy. Morphologic evidence was offered in proof that lesions of tuberculosis in experimentally infected guinea pigs may regress or actually resolve under the influence of promin.

THE INHIBITORY EFFECT OF OXYGEN ON STARVING PATHOGENIC BACTERIA. Carl E. Cahn-Bronner (by invitation), Chicago, Ill.

Abstract. Some aerobic and facultative anaerobic pathogenic bacteria were cultured under partial starvation by quantitative reduction of the nutrient content of the media.. Under such conditions bacteria multiply only when exposed to decreased oxygen tension. Atmospheric oxygen tension inhibits growth. In shake-cultures colonies are formed only in a very thin zone below the surface where the relation between nutrient content and oxygen supply is optimum. The distance of this fine layer of colonies from the surface and the depth of the zone of inhibition below the surface increases inversely with the amount of nutrient and directly with the oxygen tension. This sensitivity to oxygen varies with the temperature, with the pH and with exposure to light. The less the media contain of an easily oxidizable substance, acting as an oxygen acceptor, the more inhibitive is the effect of oxygen. This is due to peroxides formed by the bacteria in abundance of oxygen.

It could not be demonstrated that starving bacteria form more peroxides or less catalase. But it could be shown that minute amounts of hydrogen peroxide are much more stabile in starvation media which are either sterile or contain living bacteria. Some peroxides even may be formed on the surface of such sterile colloidal media by exposure to oxygen. The same phenomenon of layer formation in shake-cultures can be produced by hydrogen peroxide in rich media, as by oxygen under starving conditions. Studies on the bacteriostatic action of hydrogen peroxide revealed that the activity of hydrogen peroxide and the effect of oxygen show the same relationship to variations of both the nutrient content and the pH of the media.

EXPERIMENTAL OBSERVATIONS ON THE BACTERIOSTATIC ACTION OF HYDROXYETHYLAPOCUPREINE UPON DIPLOCOCCUS PNEUMONIAE. Mark M. Bracken and (by invitation) George E. Crum and Albert G. Corrado, Pittsburgh, Pa.

Abstract. Hydroxyethylapocupreine, a quinine derivative made at the Mellon Institute, Pittsburgh, and used in the treatment of pneumonia and malaria, was previously believed to be bactericidal for *Diplococcus pneumoniae*. *In vitro* studies had

Discussion

(Dr. Israel Davidsohn, Chicago, Ill.) Did you notice whether there was any evidence of precipitins in patients who had wound separation? It seems to me that the reason why you failed to notice any correlation between the response to catgut and the response to the guinea pig kidney is because the antigen is contained only in the sheep red cells, and I doubt whether it is contained in the serum or in any of the tissues of the sheep.

(Dr. Hopps) Titration of the serum of the patients suffering wound disruption was made only in one case, and in this case there were no antibodies for catgut discoverable. In regard to the heterophil antibody, it was thought that inasmuch as some serum protein was contained in the catgut, there might also be sheep's red corpuscles contained there in sufficient quantity so that a reaction could occur to heterophil antibody.

IMMUNOLOGIC AND TOXIC PROPERTIES OF CASEIN DIGEST AS PREPARED FOR PARENTERAL ADMINISTRATION. Howard C. Hopps and James A. Campbell (by invitation), Chicago, Ill.

Abstract. Is casein digest a safe source of amino acids for parenteral administration? To determine the antigenic or allergenic properties of such digests, rabbits and guinea pigs were sensitized to casein, swine serum and swine pancreas (swine pancreas was used in the preparation of one of the products tested). Animals were also given repeated injections of casein digest; both an acid and an enzymatic hydrolysate were used. Skin tests, precipitin determination and anaphylactic tests failed to demonstrate allergic properties in the products tested.

To determine the toxic properties of casein hydrolysates, reactions of normal smooth muscle strips to casein digests were studied. These studies indicated the presence of histamine-like substances, peptones, or tyramine which might explain the frequently observed reactions of flushing, sensation of warmth and nausea in patients to whom casein digest was administered parenterally. Hydrogen ion concentration and activity were determined as well as their effect on acid-base balance in plasma and in circulating blood. Although these substances were quite acid and markedly lowered the pH of plasma to which they were added, they caused no appreciable change in pH or CO_2-combining power in the circulating blood of human beings. This was considered as evidence of the rapid rate at which amino acids are synthesized into protein.

Evidence was presented from postmortem studies upon individuals who had received casein hydrolysate parenterally that casein digest does not alter the morphology of tissues to a significant degree in those patients who have adequate liver function. The suggestion was made that patients with poor liver function may not be able to synthesize protein at a sufficient rate to conjugate rapidly the administered amino acids and thus may suffer severe acidosis.

OBSERVATIONS ON TUBERCULOUS GUINEA PIGS BEFORE AND AFTER TREATMENT WITH SODIUM P,P'-DIAMINODIPHENYLSULFONE-N,N'-DIDEXTROSE SULFONATE (PROMIN). William H. Feldman and (by invitation) Frank C. Mann and H. Corwin Hinshaw, Rochester, Minn.

Abstract. At the 1941 meeting of the American Association of Pathologists and Bacteriologists there was described an experiment which indicated that a compound known unofficially as promin was capable of exerting a definite effect upon experimental tuberculosis in the guinea pig even though the treatment was delayed for as long as 6 weeks after the infection was introduced. The treated animals lived longer than the untreated, and in the treated animals the anatomic character of

amounts of antibody would alter the course of infection in the presence of alcoholic intoxication.

In this study 120 rabbits were used. Of these, 56 were actively immunized, 20 passively immunized and 44 were non-immune. Thirty-four of the actively immunized, 15 of the passively immunized, and 22 of the non-immune animals were intoxicated with alcohol throughout the course of the experiment. The infection was started by the intradermal inoculation of 0.1 cc. of a 6- to 8-hour culture of type I pneumococci. Antibody content of the serum was determined by the resuspension-agglutination technic. All the non-immune and the intoxicated passively immunized animals died, while only half of the intoxicated actively immunized animals died. The latter survived longer than either of the former groups. The actively immunized animals had much more antibody than the passively immunized animals. The animals of both these groups survived the infection when the intoxication was withheld. The difference between the lesions of the intoxicated and non-intoxicated animals consisted mainly in the reduced number of leukocytes and the increased number of extracellular bacteria present in the former. The reduction in number of leukocytes and the increase in number of extracellular bacteria was greatest in the intoxicated non-immune, less marked in the intoxicated passively immunized, and least marked in the intoxicated actively immunized animals where the antibody content was greatest.

Conclusions: Alcoholic intoxication reduced resistance to infection by inhibiting the rapid mobilization of the local inflammatory response; consequently the bacteria multiplied too rapidly for the reduced number of leukocytes entering the infected site to destroy them. This effect was opposed by antibody, which, unharmed by alcohol, accelerated the sluggish inflammatory response, enabling significant numbers of leukocytes to reach the area of inflammation, while the antibody effectively checked the spread of the bacteria.

Discussion

(Dr. Alvin G. Foord, Pasadena, Calif.) How much alcohol did these animals get, and what would be the corresponding dose in human cases?

(Dr. Lushbaugh) These animals averaged about 2,000 gm., and it was necessary to give them 50 cc. of 25 per cent alcohol by stomach tube in order to put them in the condition we required, that is, a stupor bordering closely on coma. The alcoholic determination of the blood showed that they had 400 to 600 mg. of alcohol per 100 cc. of blood, which in man would be "dead drunk."

(Dr. Foord) In the first chart you did not have the control of the animals who had alcohol without any pneumococci. Did any of them die from the alcohol?

(Dr. Lushbaugh) I controlled that, but did not put it in. I used 22 animals which received comparable amounts of alcohol, but no pneumococci. One animal did die of a bronchopneumonia, which none of the other animals showed, and 1 died from the effect of the stomach tube being put into its trachea. In other words, the two deaths in this group were accidental and not comparable to the deaths occurring among the immune animals that were infected and similarly intoxicated. Blood cutures showed that the latter had pneumococcic septicemia at the time of death.

THE PATHOLOGY OF SPONTANEOUS COCCIDIOIDAL GRANULOMA IN THE LUNGS OF WILD RODENTS. L. L. Ashburn and (by invitation) C. W. Emmons, Bethesda, Md.

Abstract. Emmons recently reported the finding of fungi in 25 of 105 animals trapped in the desert around San Carlos, Arizona. Of the 25 fungus-infected animals, 7 of the pocket mice *(Perognathus)*. 1 kangaroo rat *(Dipodomys)* and 1 ground squirrel *(Citellus)* showed gross lesions in their lungs at autopsy. The presented report described these lesions and the fungus associated with them.

demonstrated that no growth occurred in hormone veal broth or in poured blood agar plates inoculated with *D. pneumoniae* type II after 6 hours' incubation in broth containing a 1:2500 dilution of hydroxyethylapocupreine. The present experiments substantiate these findings, but also demonstrate that mice which were inoculated intraperitoneally with the chemical-broth-culture mixture after 6 hours' incubation of the latter, developed fatal infections. Corresponding mice which received the same dosage of chemical-broth-culture mixture after 18 hours' incubation survived. Peritoneal washings of these latter mice were cultured in poured blood agar plates and identical portions were inoculated into fresh mice at intervals of 2 to 24 hours after infection. Many of these blood agar plates showed smooth colonies of *D. pneumoniae,* but all of the corresponding mice survived.

The viability of the *D. pneumoniae* was proven by its growth on the blood agar plates inoculated with peritoneal washings of mice which survived the infection. This demonstrates that hydroxyethylapocupreine, under the conditions of these experiments, is not primarily bactericidal, but is bacteriostatic.

Survival of mice demonstrated to be infected with viable *D. pneumoniae* suggests some reduction of invasiveness or pathogenicity of *D. pneumoniae* after exposure to hydroxyethylapocupreine. It is to be noted that the strain of *D. pneumoniae* used in these experiments uniformly produced fatal infection in mice which were inoculated with one to three microörganisms not treated with hydroxyethylapocupreine in other experiments. Loss of pathogenicity, if such it was, was not maintained. After passage through broth of these "avirulent" *D. pneumoniae,* larger doses produced uniformly fatal infections in primarily healthy mice.

Finally, the experiments demonstrate that bactericidal action shown by *in vitro* methods may in reality be bacteriostasis when studied *in vivo.*

Discussion

(Dr. Howard H. Permar, Pittsburgh, Pa.) I feel that Dr. Bracken has been very wise in employing the word bacteriostatic, and not enlarging on that, because were we to go into virulence, invasiveness and pathogenicity, we might very well get lost. I think he has shown *in vivo* that this chemical has a remarkable effect upon a virulent pneumococcus when transplanted from "primary" to "secondary" mice, at the intervals indicated. Furthermore, it may be that this method could be employed in the study of chemotherapeutic agents which are now being used, and some information of value might be brought forth.

(Dr. M. H. Soule, Ann Arbor, Mich.) Dr. Bracken, do you know whether or not this compound has been used in the treatment of human malaria? The compound mentioned by Dr. Feldman—promin—has been used with rather encouraging results, and inasmuch as we need a substitute for quinine, I am wondering whether the rather optimistic results reported by Dr. Hegner on avian malaria have been confirmed in the human disease.

(Dr. Bracken) This compound has been used in one case of human malaria. The patient was admitted to Mercy Hospital with recurrent chills and fever, and diagnosed as tertian malaria. At the time of the second chill in the hospital, hydroxyethylapocupreine therapy was instituted at a dosage of 120 gr. a day, 15 gr. every 3 hours. The temperature then dropped to normal, and remained normal for the duration of his hospital stay of about 12 days. That was 2 years ago. Since that time there has been no recurrence, so far as I know. This chemical is under observation in the treatment of malaria by the United States Army in the Panama Canal Zone.

THE EFFECT OF ALCOHOLIC INTOXICATION UPON ACQUIRED RESISTANCE TO PNEUMOCOCCAL INFECTION IN RABBITS. Clarence C. Lushbaugh (by invitation), Chicago, Ill.

Abstract. This study was undertaken in an effort to determine (1) if alcoholic intoxication might influence the mechanisms of active immunity, and (2) if large

were obtained by smearing the cut surface of the lung on the culture media. From this it appears that the cultures obtained were from the extra-granulomatous fungus cells.

Mediastinal lymph nodes were seen microscopically in only one animal and this showed no involvement. As to the rest of the body, we examined the pancreas, liver, spleen and kidney in about half of the group, and they were negative grossly and microscopically.

FACILITATION OF INFECTION WITH EQUINE ENCEPHALOMYELITIS VIRUS. Lester S. King, Newtown, Conn.

Abstract. Fifty per cent glycerin injected intraperitoneally, intramuscularly, or intravenously greatly increases the virulence of fixed equine encephalomyelitis virus injected intramuscularly. This increase is approximately 100 times. Evidence was presented that this result is brought about through dehydration, suddenly produced. Gradual dehydration to the same degree, produced by depriving animals of drinking water, is without effect on virus action. The facilitation effect can be produced by injections of very concentrated sodium chloride, but not by distilled water or by 5 per cent glucose. Glycerin has no facilitating effect when the virus is administered intranasally or intra-ocularly

ATTEMPTS TO INFECT VARIOUS STRAINS OF COTTON RATS *(Sigmodon)* WITH THE VIRUS OF POLIOMYELITIS. Marjorie E. Pierce, J. Emerson Kempf (by invitation), and Malcolm H. Soule, Ann Arbor, Mich.

Abstract. A report by Armstrong in 1939 cited the successful transmission of poliomyelitis to the eastern cotton rat *(Sigmodon hispidus hispidus).* Within 3 months the author had adapted this rat strain to white mice. The importance of having a cheap susceptible laboratory animal for the study of the many unsolved problems of infantile paralysis directed the efforts of many investigators to confirm this work. Our group was most fortunate in having the coöperation of expert mammalogists from the Laboratory of Vertebrate Genetics of the University of Michigan in identifying and obtaining a supply of cotton rats. Armstrong had experienced considerable difficulty in establishing the initial infection in the rodents and it seemed possible that age and species might be important factors. Accordingly, animals were trapped in Florida, Alabama, Louisiana and Arizona. All belonged to the genus *Sigmodon* and were identified as: *hispidus hispidus, hispidus littoralis, hispidus cienegae* and *minimus minimus.* Fertile pairs were mated and breeding colonies established to supply animals of known ages. Those raised in the laboratory have in no manner become domesticated; they are vicious and difficult to handle.

In the experimental work, trapped animals and pedigreed young were treated with suspensions of human cord from seven patients dead of typical bulbar poliomyelitis and monkey cord infected with six human strains well adapted to this host. The infected material was administered intracerebrally, intranasally and subcutaneously to each animal. One group was given one treatment and carefully observed for a period of 90 days. A second group was treated in a similar manner and at the end of 7 days the animals were sacrificed, the brains and cords removed and a new series of rats were treated with suspensions of this material. The procedure was repeated several times in an attempt to adapt the various strains to the rats by serial passage. A third group was treated with virus suspended in the "enteric toxin filtrate" of Toomey. At no time was flaccid paralysis observed in any of the treated animals and the histological examinations made of cord preparations showed no evidence of poliomyelitis.

Microscopically, a total of 20 nodular lesions was found in the 7 mice and 1 kangaroo rat. These lesions occurred most often in the lower lobes and particularly along the anterior border and in that portion of the lung occupying the costophrenic angle. Most of them were superficially located and many caused slight elevation of the overlying pleura. Except for 1 animal, most granulomata measured approximately 1 mm. in diameter. Basically they were formed of fusiform epithelioid cells diffusely and irregularly disposed in the center of the nodules, but often showing concentric arrangement peripherally. A few lesions were formed centrally of adherent, large mononuclear cells. Six granulomata had large central zones of caseous necrosis, varying from 300 to 800 μ in diameter. One such area of necrosis was largely calcified. Fibrosis was present in only 2 lesions, being of moderate degree in 1, and slight in the other. A few lymphocytes were present in the outer wall of most granulomata and 3 had sharply limited, densely cellular lymphocyte mantles varying from 25 to 200 μ in thickness. The multiple small pulmonary lesions present in the ground squirrel were formed of grouped alveoli filled with sheets of large mononuclear cells. In all granulomata there were present few to many fungus cells of varying size. In 2 granulomata (2 mice), 6 fungi showed cytoplasmic cleavage or mature endospores, typical of coccidioides.

Discussion

(Dr. C. L. Davis, Denver, Colo.) It is quite interesting to note that in these rodents the lesions were confined to the thoracic cavity. That is comparable to the 77 cases in cattle studied in the Denver pathological laboratory of the U. S. Bureau of Animal Industry. In no instance have we thus far found coccidioidal invasion outside the thoracic cavity. I am beginning to wonder now, in the light of this information, if the animal species does not have some sort of body resistance to the extent of confining this disease to the thoracic cavity. It is quite different in man, where lesions are likely to be found in any part of the body.

(Dr. Ernest M. Hall, Los Angeles, Calif.) I would like to know if there were any lesions in the tracheobronchial lymph nodes.

(Dr. Alvin G. Foord, Pasadena, Calif.) I think it is of practical interest to us who make examinations of sputum or lung tissue in suspected cases that we should realize if we treat the material with sodium hydrate or acid, as we do tuberculous material, to kill the other organisms so that they will not kill the guinea pig, we will kill the coccidioidal granuloma organism. Smith, who did so much work at Stanford University, found he could kill the streptococci and other organisms in lung tissue and sputum by 0.5 per cent copper sulfate, which allows the coccidioidal spherules to persist, and then by injecting that material and making cultures he was able to obtain the organism. I am sure that in our part of the country we have missed making the diagnosis in the past because we have treated sputum, etc., like tuberculous sputum, killing the coccidioidal organism by so doing.

(Dr. George H. Hansmann, Milwaukee, Wis.) Are you certain that the coccidioides described is the same as that in man?

(Dr. Ashburn) To answer the last question first, the fungus cultures obtained from these animals were not discussed because this phase of the study is incomplete. However, Dr. Emmons obtained *Coccidioides immitis* in culture from 3 of the mice trapped early in the study. These showed no gross lesions and were not saved for histologic examination. These fungi have been inoculated into experimental animals and the pathology is typical of that produced by strains from human cases. In addition to the 3 mice mentioned, fungi were also found in 22 other animals. These fungi were not *C. immitis*. In all of the animals discussed here, there were extra-granulomatous fungus cells, differing slightly from those within the nodules, mainly in their uniform size and staining reaction. The cultures

duttoni; viruses of eastern equine encephalomyelitis, rabies, poliomyelitis, influenza, vaccinia and *Bacillus coli* bacteriophage; *Trypanosoma lewisi, Trypanosoma brucei, Trypanosoma hippicum* and *Trypanosoma equiperdum.* Before placing the specimens, blood or tissue suspensions in the refrigerator, each was distributed in lusteroid vials previously sterilized by ultraviolet light and quickly frozen in thin layers on the inside of the tubes by immersion in CO_2 and C_2H_5OH at −52° C. At definite intervals following storage, samples were selected and promptly thawed by placing at 37° C. Microscopic examinations were made of the suspensions and animals were inoculated to determine any change in virulence. All of the germs survived and retained their original potency. Certain pronounced morphologic changes were noted in the trypanosomes and to some extent in the spirochetes. The longest storage period evaluated at the time of this report is 148 days. However, the experiment is still in progress and there is no reason to believe the data will be materially altered by subsequent tests. It is apparent that a temperature of −52° C. is just as satisfactory for the preservation of microbic forms as −76° C. The low temperature electric refrigerator has many advantages over the carbon dioxide chest for this purpose.

Discussion

(Dr. S. R. Haythorn, Pittsburgh, Pa.) I should like to ask Dr. Soule if he has tried to preserve *Treponema pallidum* at −51° C.?

(Dr. Soule) Turner was the pioneer in this particular type of work with *Spirochaeta pallida,* and we have confirmed his experiments in every way. We ground up infected rabbit tissue, froze the suspensions quickly and then stored them at −51° C. This material produced typical infections in rabbit testes. We have found a temperature of −50° C. just as satisfactory as −76° C., the temperature used by Turner, for the preservation of syphilitic material.

(Dr. William H. Feldman, Rochester, Minn.) Have you applied this method to any of the strains of mycobacteria?

(Dr. Soule) The mycobacteria are so easy to culture and maintain that we have not applied this method to them. The problem we have is with trypanosomes, spirochetes and viruses—forms hard to cultivate. If we get a group of viruses, we like to have them kept in a manner which will maintain their virulence just about at the level at which we received them.

(Dr. William H. Harris, New Orleans, La.) Dr. Soule spoke about the altered morphology of the trypanosomes after freezing, and yet referred to their subsequent virulence in animals. The original morphological characteristics most likely reappeared on re-injection in animals, did they not?

(Dr. Soule) Yes.

(Dr. Harris) Then their nature is not really changed by this process?

(Dr. Soule) That is a problem that has bothered us, particularly with the protozoa. There is no reason to believe that we change their nature by the freezing process. With malarial blood the changes are not great.

(Dr. Harris) Then it is not a really serious problem? The process of freezing, after all, really answers the primary purposes of preservation, inasmuch as there occurs restoration of all the biological characteristics through subsequent animal inoculation.

(Dr. Soule) It would be more encouraging if we could control the morphological changes in the freezing and thawing processes. It is extremely important to freeze very rapidly.

These negative results suggest that the success of Armstrong was not related to a susceptibility associated with age or species. It would seem more probable that the strain which he established in cotton rats after preliminary adaptation to monkeys is a most unusual strain of the virus of poliomyelitis. In the present state of our knowledge the cotton rat offers no advantage over other rodents as an experimental animal for the study of this disease agent.

Discussion

(Dr. William H. Harris, New Orleans, La.) In connection with his experiments, I would like to ask Dr. Soule if he has had any experience with the murine infection that has been stated to be of spontaneous origin and is quite analogous to poliomyelitis. Furthermore, I would add that we have worked with the murine strain from a primary human source which Dr. Jungeblut has employed in his experiments. It was difficult to transmit when received in ordinary shipment, until finally he kindly sent it air mail and in dry ice. With this latter specimen we were successful in carrying that strain along. The lesions of these animals are quite similar to those of poliomyelitis.

(Dr. Soule) After seeing the histological sections of Dr. Jungeblut at Chicago last spring, we are not convinced he is dealing with a strain of poliomyelitis in his animals. The only strain which we feel confident is entitled to consideration as a cotton rat-adapted strain of poliomyelitis is the Lansing strain of Armstrong.

(Dr. Harris) Are you familiar with the spontaneous disease?

(Dr. Soule) No.

(Dr. Harris) I must state that so far as we can ascertain, the clinical signs and pathological changes of poliomyelitis were present in our animals.

(Dr. Soule) Are you not referring to the strain of poliomyelitis which Dr. Jungeblut claims he has adapted to mice?

(Dr. Harris) Yes; I primarily asked the question concerning the spontaneous disease and next discussed the Jungeblut-adapted strain.

THE PRESERVATION OF BACTERIA, VIRUSES AND PROTOZOA AT —51° TO —53° C.

Malcolm H. Soule and (by invitation) Ruth Lofgren and Marjorie E. Pierce, Ann Arbor, Mich.

Abstract. Solid carbon dioxide (dry ice) is quite widely used as a refrigerant for the preservation of disease agents such as viruses, spirochetes and protozoa; forms which cannot be cultured readily on artificial media. In actual practice the dry ice is mixed with ethanol and stored in vacuum flasks along with vials of the germ specimens, or used in a manner similar to ice in specially designed chests. With constant replacement of the refrigerant a temperature of —76° C. may be maintained indefinitely. When adequate attention is given to the freezing and thawing of the infectious material, the microbes not only remain viable but the original virulence is retained to a marked degree. The major objection to this technic is the problem of handling and providing a supply of dry ice. In addition the carbon dioxide may have a toxic action on the germs. Recently a commercial low temperature electric refrigerator (Deep-Freeze) for home use with a capacity of 4 cubic feet came on the market. With the coöperation of the manufacturers one of the machines was constructed with a special mechanical unit and insulation, resulting in the obtaining of an operating temperature of —50° to —53° C. This instrument has been in continuous use for over 12 months and the temperature has not varied more than 2 degrees. Suspensions of infected material containing the following organisms have been placed in the refrigerator: *Spirochaeta novyi, Spirochaeta obermeieri, Spirochaeta kochi* and *Spirochaeta*

psittacosis of pigeon origin showed a similar increased pathogenicity for pigeons by the intracerebral route, as compared with ten strains of psittacosis viruses of parrot origin. The viruses of parrot origin, however, commonly produced latent infection in pigeons even when clinical illness was not evidenced.

For the isolation of psittacosis of pigeon origin from human sputum, the intracranial injection of mice or pigeons may be essential, although it is probable that the intranasal injection of mice would be successful. The intraperitoneal injection of mice may give negative results.

The virus of lymphogranuloma venereum is apparently non-pathogenic for pigeons by the intracerebral route.

POSTMORTEM EVIDENCE OF ACUTE INFECTION IN UNEXPECTED DEATH IN INFANCY.
Jacob Werne, New York, N. Y.

Abstract. This presentation emphasized the occurrence of pulmonary lesions in a group of infants dying unexpectedly, in whom the gross autopsy findings were inadequate for the reasonable certification of death. In 50 such cases collected over a 10-year period from a routine medicolegal autopsy service in the Borough of Queens (in the Office of the Chief Medical Examiner of New York City), bacteriological study was possible in 28. The histological findings were sufficiently uniform in all to warrant the inclusion of the remaining 22 cases.

The organisms encountered, and the number of times, were as follows:

Streptococcus viridans	12
Streptococcus, non-hemolytic	9
Bacillus coli	9
Staphylococcus aureus, non-hemolyzing	8
Streptococcus hemolyticus	7
Staphylococcus aureus, hemolytic	3
Streptococcus liquefaciens	2
Streptococcus faecalis	2
Streptococcus foetidus	1
Salmonella derby	1
Brucella melitensis	1
Friedländer's bacillus, A	1
Pneumococcus, type 14	1
Pneumococcus, type 21	1
Pneumococcus, not type-specific	1

The majority of sections of lung showed the occurrence of bronchitis, peribronchitis and peribronchial pneumonitis; mononuclear cells predominating. Extreme capillary congestion, pericapillary and intra-alveolar edema, and edema and cellular infiltration of subpleural and larger septal tissues were frequently found. In a smaller number of cases the presence of a diffuse interstitial reaction without evidence of primary bronchial inflammation suggested other than a respiratory portal of entry. Such was the picture in the cases showing dermatitis and in one where the presence of a microscopic pylephlebitis suggested an intestinal portal of entry.

Taken in conjunction with postmortem bacteriological findings, the presence of the lesions described, illustrated by lantern slides, supports the conclusion that acute infection was the cause of death in these cases.

Discussion

(Dr. Howard C. Hopps, Chicago, Ill.) In one of the sections shown there appeared to be macrophages filled with large fat droplets. Was lipoid pneumonia present in these cases?

THE HISTOLOGY AND TOPOGRAPHY OF THE BRAIN LESIONS OF EQUINE ENCEPHALOMYELITIS IN MAN. James H. Peers, Washington, D. C.

Abstract. In the summer of 1941 a severe epidemic of encephalitis occurred in North Dakota and neighboring states. The following description of the pathology of this disease was based upon examination of 13 brain specimens, in 4 of which the virus of the western type of equine encephalomyelitis was demonstrated by animal inoculation. This virus may produce in man a widespread and severe encephalitis, characterized by nodular microglioses, spongy areas of partial disintegration of the ground substance and perivascular round cell infiltration. These lesions constantly appear in greatest number and severity in the anterior part of the putamen and caudate nuclei, and markedly diminish posteriorly. In the brain stem, lesions are evenly distributed in the tegmentum and base, apparently independent of anatomic and functional units. A variable number of lesions are also present in the anterior cerebral cortex, and in the cortex and the roof nuclei of the cerebellum. The disease process is predominantly located in the gray matter, but scattered foci of microgliosis with partial demyelinization are seen in the white matter. The meninges contain only a scanty round cell infiltration. No inclusion bodies were observed.

Discussion

(Dr. Lester S. King, Newtown, Conn.) I think it rather remarkable that the histological sequence which Dr. Peers describes differs so extremely from what is found in the eastern type of equine encephalomyelitis. I have had considerable opportunity to study lesions in horses, and experimentally in guinea pigs and mice, and in human material. In the eastern type of the disease, the characteristic lesion is a focus of polymorphonuclear leukocytes, sometimes around a vessel, and sometimes diffusely through the tissue, independent of a vessel. In addition, the blood vessels often show marked inflammatory changes in their walls. The glial nodules are prominent in this type also. The spongy areas which appear in the western type are not so prominent in the eastern type, and where they do occur, they appear to be independent of any inflammatory change. From the differences in pathology we cannot draw any conclusion as to the similarities or relationship of the two viruses. That can only be determined by serological and immunological work, but I wish to emphasize again that the types of pathology are quite different in the disease which Dr. Peers has discussed and in the eastern disease with which I am familiar.

(Dr. Peers) I want to thank Dr. King for bringing out these points. I, myself, have had no opportunity to see any of the eastern material. I have had some promised me, and I shall be glad to look for these differential features which Dr. King mentioned.

COMPARATIVE STUDIES OF MENINGOPNEUMONITIS VIRUS PSITTACOSIS OF PIGEON ORIGIN AND PSITTACOSIS OF PARROT ORIGIN. H. Pinkerton and (by invitation) V. Moragues, St. Louis, Mo.

Abstract. Comparative morphological, histological and biological studies suggest a close relationship between the meningopneumonitis virus of Francis and Magill and a virus recovered by Pinkerton and Swank from thiamin-deficient pigeons. Both of these viruses are morphologically identical with typical psittacosis, and it seems probable that they are biologically modified strains of psittacosis. They both differ from typical psittacosis in that they show much greater pathogenicity for the pigeon after intracranial injection, and fail to produce hepatic necrosis after intraperitoneal injection in mice. A virus recently isolated from human cases of atypical pneumonia by Eaton, Beck and Pearson behaves similarly in mice, and may also be closely related to these two viruses. Six other strains of

appeared that these children were normal. The bright red color of the lungs shown and the emphasis upon lymphoid cell distribution and edema in the interstices appear to me to be matters of observation rather than definite explanation.

(Dr. Werne) The time has limited my presentation to a discussion of the pulmonary lesions, which I consider the most apparent. Many of my cases show glomerular lesions, foci of cellular infiltrate in heart and liver, and extreme hyperemia and hyperplasia of lymphatic tissues. These will be reported in a subsequent presentation.

(Dr. Seaton Sailer, Cincinnati, O.) Is this prominent interstitial edema which has been described regarded as a serous inflammatory exudate, or is it a cold process—a simple edema following the shock of overwhelming toxic absorption?

(Dr. Werne) I believe that several factors are involved. We have direct evidence in some of these cases of bacterial injury in the form of capillaries distended with bacterial colonies. In others we find, as illustrated, bacterial colonies in the bronchioles. In association with both types of occurrence there is a significant reaction. Extreme capillary congestion, pericapillary and intra-alveolar edema and marked edema of the subpleural and larger septal tissues are noted. The association with this edema of significant cellular infiltration, mononuclear in the main, speaks for an inflammatory origin, I believe. Part of the picture is certainly referable to the edema that we now recognize as a histopathological expression of shock. The shock in this instance, I believe, is referable to the fulminating bacterial process.

(Question from audience) How many hours after death were the autopsies done, and were any cases used as controls?

(Dr. Werne) In most instances the bodies were refrigerated for 4 to 6 hours after death, and the autopsies performed either the same day or the following morning. We have been controlling our bacteriological findings in this group by routinely culturing the viscera of infants and children dying of violence, and in the last four cases, which happened to be drownings, the lungs were sterile in all but one instance, in which *Staphylococcus albus* was recovered. There were no visceral lesions in these control cases.

MEDIASTINAL SYMPATHOGONIOMA. Seaton Sailer, Cincinnati, O.

Abstract. A colored female, 65 years old, was admitted to the accident room of Cincinnati General Hospital in coma. Three days before admission she had complained of severe headache, dizziness and weakness. Upon admission, spinal fluid cultures and smear were positive for *Haemophilus influenzae*. Death occurred 2 days later and autopsy showed an extensive purulent leptomeningitis involving the entire brain and spinal cord. Some bronchopneumonia was present in the left lower lobe. Occupying the anterior superior mediastinum and overlying and bound to the superior vena cava by fibrous adhesions, was a well encapsulated, solid, firm tumor 5 cm. in diameter. Portions of this were also attached to the mediastinal surface of the right lung and the eparterial bronchus by fibrous bands. On section the tumor capsule was thick and partially calcified. The parenchyma was reddish brown and rather soft. Microscopic sections showed the histologic structure of a sympathogonioma with closely packed, deep-staining, small, round and oval cells showing some attempt at rosette formation. There was no evidence of metastasis. The few cases of this condition found in the literature were briefly reviewed.

NEUROBLASTOMA OF THE MEDIASTINUM CONTAINING PHEOCHROMOBLASTOMATOUS ELEMENTS. H. R. Wahl and (by invitation) David Robinson, Kansas City, Kans.

Abstract. A brief review of sympathetic nerve tumors arising in the thorax was given with report of a case occurring in a child of 4 years. Forty-one related

(Dr. Werne) The section in question shows very marked pericapillary edema, and I believe the droplets are hydropic rather than fat. In many of these cases routine fat stains were done of lung and liver. In no instances was evidence of lipoid pneumonia or reaction to foreign body found.

(Dr. E. T. Bell, Minneapolis, Minn.) I am glad to hear Dr. Werne's paper. I think if more studies like this were made we would seldom be called upon to make the diagnosis of status lymphaticus.

(Dr. Werne) I agree with Dr. Bell as to the inadvisability of making a diagnosis of status lymphaticus. I recognize hyperplasia of lymphatic tissues and the presence of an uninvoluted thymus solely as anatomic findings, but not in themselves as adequate explanation for death.

(Dr. Wiley D. Forbus, Durham, N. C.) I think this a very important subject from the medicolegal point of view. Almost all of us have had the experience of being called to explain such deaths in children as have been described in this paper. If one is not familiar with the rapidity with which death can result from overwhelming bacterial infection, one may find himself in great difficulty and embarrassment in dealing with cases of "sudden death" and be led quite far astray by incidental autopsy findings that would be wholly ignored under different circumstances. This presentation is a timely one.

(Dr. O. O. Christianson, Peoria, Ill.) This paper interested me very much because lately I had a similar case. A child of 3 months had received an overdose of atropine because the druggist had made an error in filling the prescription. Two days later the child became very ill and died. Findings at autopsy were quite negative except for what was considered to be terminal aspiration pneumonia. However, I was surprised to find that cultures of the lungs, spinal fluid and blood revealed a heavy growth of *Streptococcus viridans*. The histologic changes in the lungs were identical with those described by Dr. Werne. In this case the parents' lawyer is attempting to prove that the child died from an overdose of atropine. However, I am certain that the autopsy findings will frustrate this attempt.

(Dr. George H. Hansmann, Milwaukee, Wis.) I wonder why this is limited to children; it happens in adults, too, with overwhelming infection. One is often discouraged in routine autopsies to find the blood cultures are not very valuable, but as here demonstrated, they are of occasional importance. On the other hand, I wonder what the mechanism is? Do some of these children die of asphyxia due to the aspiration of milk? I wonder if anoxemia sufficient to cause asphyxiation occurs from the aspiration of milk or food which had been regurgitated. I noted that bile was found in the terminal bronchioles.

(Dr. Werne) Cases of fulminating infection are also met with in adults, but far less frequently than in children, and very rarely without some gross evidence upon the basis of which death may be reasonably certified. For that reason their occurrence constitutes a relatively minor medicolegal problem and was not considered in this presentation. With regard to the mechanism of death, there are some in whom aspiration of stomach contents is either a terminal or a significant terminating phenomenon. In the majority of cases the pulmonary edema must be regarded as the strongest evidence of a mechanical basis for death. The quantitative measurement of the influence of shock, for which the bacterial findings with their associated lesions are evidence, is a difficult one with postmortem material.

(Dr. William H. Harris, New Orleans, La.) I did not hear any description nor did I see any illustration of other organs in these cases. Was the entire pathology present limited to the lungs? Prior to unexpected death, were these children regarded as entirely normal? I ask this because while a death may be unexpected, the subject may be, of course, primarily abnormal. From what I could hear, it

(Dr. Humphreys) It was not. The only case in which that has been done was that of Beer, King and Prinzmetal (Beer, Edwin; King, F. H., and Prinzmetal, M. *Ann. Surg.*, 1937, 106, 85–91), unless others have been reported quite recently.

(Dr. Howard T. Karsner, Cleveland, O.) It seems to me that the preceding three papers belong in a group and it would be unfortunate if they were not discussed. The mediastinal sympathogonioma reminds me of the case of Frost and Wolpaw (Frost, T. T., and Wolpaw, S. E. *Am. J. Cancer,* 1936, 26, 483–492) in which a tumor in the superior pulmonary sulcus with Horner's syndrome proved to be a sympathogonioma rather than the customary carcinoma.

Dr. Wahl modestly made no reference to the fact that in 1914 he published a paper on neuroblastoma which to my mind was a masterful presentation of the subject at that time. The tumor he reported today illustrates the fact that tumors of the sympathetic system may show departure from cell type. Indeed, multiple sympathetic tumors may be of different orders of differentiation in different situations.

Pheochromocytomas may also show variations in cell picture. I should like to have Dr. Humphreys express her views about the following idea. In these tumors of the adrenal there are usually cells which, because of arrangement and of lipid content, appear to represent adrenal cortex. I have not read of, or seen, these cortex-like cells in pheochromocytomas of the thorax or those situated in the region of Zuckerkandl's organ. It is unfortunate that so many patients with tumors of endocrine organs are operated upon without having an assay of hormones. Of course the manifestations of pheochromocytomas are like those due to a large dose of adrenalin. My recollection is that although Prinzmetal demonstrated a pressor substance in the blood, he did not prove it to be epinephrine. The marked drop in blood pressure that is likely to occur as these tumors are removed at operation points strongly to the production of a pressor substance by the tumors. In one of our cases, the patient received continuous infusions of adrenalin for nearly 18 hours before he maintained blood pressure above shock levels. Evidently it took this time before the opposite adrenal (presumably atrophic) functioned adequately. The diagnosis of these tumors and the effects of their removal are a tribute to the achievements of modern medicine.

(Dr. George A. Walker, Kansas City, Kans.) Dr. Karsner referred to the fact that these pheochromocytomas sometimes contain cells suggesting an origin from the adrenal cortex. We saw a case recently in Kansas City, a child of 3 years, who had as the outstanding clinical manifestation of the disease a very well marked sexual precocity, with the adult type of distribution of axillary and pubic hair, and adiposities of the female type. Associated with these was the usual picture of paroxysmal hypertension. Air injection was carried out and revealed a tumor in the region of the adrenal gland. This was removed surgically. This patient also showed a very marked drop in blood pressure during the operation and was carried through by the administration of chloride and adrenalin. In the months that have passed since the operative removal of this tumor, the hypertension has receded; the patient has shown no instability of the blood pressure and there has been a gradual recession of the precocious sexual development. Therefore, this patient had a tumor whose histology was that of a pheochromocytoma but in addition to the usual paroxysmal hypertension associated with such tumors, this patient had also well marked precocious sexual development.

(Dr. Humphreys) One of the things I would like to speak about is the occurrence of the cortical types of cell. I am quite sure there are cells in at least two of the tumors which I illustrated which look very much like cortical cells. I believe Dr. Weller pointed out some time ago (quoted in Coller, F. A.; Field, Henry, Jr., and Durant, T. M. *Arch. Surg.*, 1934, 28, 1136–1148) that in a tumor he had studied some of the cells very closely resembled those of the cortex.

tumors were found in the literature, including 27 ganglioneuromata, 13 neuroblastomata and 2 paragangliomata. This tumor involved the upper right mediastinum with direct extension into the upper right lobe and metastasis to the cervical lymph nodes and to bones, especially the skull. The main tumor mass was composed of both undifferentiated and differentiated elements. The former were neuroblasts or sympathicoblasts and formed metastases to the bones. The latter were larger, had more cytoplasm and formed a tissue that was partly encapsulated and simulated that frequently described in the medulla of the adrenal gland as a paraganglioma or pheochromocytoma. This portion of the tumor showed many giant cells, often multinucleated. An argentaffin reaction was obtained but the chromaffin reaction was inconclusive, probably because of unsatisfactory fixation. A few immature ganglion cells were present.

Of the three types of sympathetic nerve tumors reported, various mixtures have often been noted. The pheochromocytoma tends to be found in the pure state more often than either the ganglioneuroma, the neuroblastoma, or its more differentiated form, the sympathicoblastoma. This case is unique in that a large part is definitely undifferentiated and malignant and made up of neuroblasts and sympathicoblasts, while the differentiated portion, instead of showing the usual ganglion cells and nerve fibrillae, is made up of cuboidal, polyhedral and giant cells such as are seen in the true pheochromocytoma.

PHEOCHROMOCYTOMAS: MORPHOLOGY AND FUNCTIONAL ASPECTS (A STUDY OF FOUR CASES). Eleanor M. Humphreys, Chicago, Ill.

Abstract. The pheochromocytomas studied were found in the adrenal glands of four women whose ages were 51, 47, 41 and 36 years. They demonstrated well the pathologic physiology and morphology of this interesting endocrine tumor. In one case, a fall initiated a fatal attack. Although this woman's main symptoms were like those of shock, her systolic blood pressure fluctuated between 130 and 268 mm. of Hg for 48 hours, and terminally her temperature reached 109° F. The only noteworthy findings at autopsy were the small tumor and an oblique crack in the neck of one femur. Two of the other patients had been near death from pulmonary edema. All three had attacks associated with paroxysmal hypertension, abolished by excision of their tumors. Most of the manifestations of attacks were strikingly like those effected in man and animals by suitable doses of adrenalin. One exception was bradycardia, which often replaced or alternated with the expected tachycardia. It was interesting that two of the patients had hypersensitive carotid sinus reflexes of the vagal type.

One case illustrated a common diagnostic problem. Thyrotoxicosis was suspected because of the patient's nervousness and vasomotor instability, her exophthalmos, and basal metabolic rates from plus 30 to plus 70. All four tumors were encapsulated and quite vascular. One was cystic; another focally necrotic and hemorrhagic. They weighed from 13.5 to 75.0 gm. Slices immersed in 3 per cent potassium dichromate solution quickly changed from gray-pink to brownish black. Assays (chemical and biological) indicated the presence of from 0.6 to 3.0 mg. per gm. of adrenalin-like substances in various samples.

Microscopically the cell types varied so greatly, even within one and the same tumor, that morphologic criteria for identification had limited value. The best criteria were the gross and microscopic chromaffinity, the assays, and, in three of the cases, the abolition of attacks once the tumor was excised.

Discussion of Papers by Drs. Sailer, Wahl and Robinson, and Humphreys

(Dr. Jack D. Kirshbaum, Chicago, Ill.) Was an adrenalin-like substance demonstrated in the blood before operation in any of these cases?

of the adrenal cortex. The first had symptoms for 4 months and died after resection of a carcinoma which had metastasized to the lungs and liver. A small adenoma was found at the autopsy of the second patient, who had been ill for 3½ years. Neither one had other endocrine tumors or hyperplasias. However, the hypophysis of each contained hyalinized basophils of the type described by Crooke, and rarely observed save in patients with the Cushing syndrome. Both women had characteristic obesity, facial hirsutism, prominent eyes, purple abdominal striations, husky voices, hypertension and amenorrhea without masculinization of the genitalia. Obesity was less typical in the previously overweight older woman, and became less striking before the other woman died. Chemical examination of the blood of the patient with the briefer illness demonstrated impaired glyconeogenesis; increased sodium and decreased potassium and chlorides, and alkalotic values for CO_2 and pH. As pointed out by others (McQuarrie, Irvine; Johnson, R. M., and Ziegler, M. R., *Endocrinology,* 1937, 21, 762–772, and Willson, D. M.; Power, M. H., and Kepler, E. J., *J. Clin. Investigation,* 1940, 19, 701–707), this electrolyte pattern is, save for the chlorides, the reverse of that found in Addison's disease. The younger patient had had symptoms for 2 years when first seen, and her subsequent course was that of progressive renal failure terminating with uremic acidosis. Autopsy findings were generalized arteriosclerosis; contracted kidneys; marked skeletal rarefaction with deformities from compression and fractures, and atrophy of endocrine organs, including the non-neoplastic parts of the adrenal cortices. This cortical atrophy and the hyalinization of hypophyseal basophils were the endocrine lesions shared by both patients. Both the carcinoma and its metastases and the adenoma had regions closely resembling the normal adult adrenal cortex. Mitoses were numerous in the carcinoma and brown pigment like that of the reticular zone was abundant in the adenoma.

Discussion of Papers by Drs. Ravid and Humphreys

(Dr. Robert C. Grauer, Pittsburgh, Pa.) I would like to ask Dr. Ravid and Dr. Humphreys whether urinary assays were done to determine the relative proportion of dehydro-iso-androsterone and androsterone. This may help to differentiate the two types of adrenal cortical tumor; the one that expresses itself entirely as an adrenogenital syndrome, from the adrenal cortical tumor which produces the manifestations of the Cushing syndrome. We know that there is a normal relative proportion of dehydro-iso-androsterone to androsterone of about 5 per cent to 95 per cent. If an adrenogenital syndrome supervenes as a result of a cortical tumor, that proportion is distributed so that there is about 40 per cent dehydro-iso-androsterone and 60 per cent androsterone excreted. Where we are confronted with a confusing tumor, one in respect to which the clinical manifestations are at variance with the pathological findings, it might be of great value to determine the relative proportions of dehydro-iso-androsterone and androsterone.

(Dr. Ravid) No assays for the androgenic hormones were done, but only for estrin and prolan, and these were negative.

(Dr. Humphreys) The first case, that of the woman with the benign tumor, was studied some years ago (1935) and the only assays for hormones were made on the urine well after the onset of the syndrome, when the patient was developing renal insufficiency. There was no variation from normal in regard to the estrogenic or male sex hormones (10 gamma per day of theelin and 14 international units of male hormone). The other case is a very recent one, and I know that a part of the tumor was to be assayed.

(Dr. Grauer) I meant urinary assay; not assay of the tumor itself.

(Dr. Humphreys) I believe urinary assays are being done.

However, it is not always easy to differentiate cortical cells, lipoid-filled macrophages and medullary cells. In one of these tumors one section had definitely chromated cells which in non-chromated sections looked like cortical cells.

As for the statement by Beer, King and Prinzmetal about epinephrine, they were naturally extremely cautious. They used the denervated rabbit's ear perfusion method and the reaction was blocked by ergotamine. On the other hand, further evidence was provided by the actual recovery of pure epinephrine from a tumor by Kendall or someone in his group at the Mayo Clinic (Kelly, H. M.; Piper, M. C.; Wilder, R. M., and Walters, Waltman. *Proc. Staff Meet., Mayo Clin.*, 1936, 11, 65–70.)

It is unfortunate that in the blood of the patients of this report the demonstration of adrenalin was not accomplished. An attempt was made by Dr. Louis Leiter in one case, without success. However, the patient on whom this attempt was made had only occasional attacks. I think if the blood of the patient whose chart I presented, showing the persistent mild hypertension and multiple daily attacks, had been investigated, we might have been more successful.

CORTICAL CARCINOMA OF THE ADRENAL WITH ADRENOGENITAL SYNDROME ASSOCIATED WITH AN ADENOMA OF THE PITUITARY. Jacob M. Ravid, Brooklyn, N. Y.

Abstract. A girl, aged 13½ years, was admitted to the Israel Zion Hospital with an 8 months' history of amenorrhea, hirsutism, pain in the abdomen and change in voice. Examination revealed obesity, coarse hair over the face and body, hypertrophied clitoris, a mass in the right lower quadrant and hypertension. On operation a mass was removed from the site of the right adrenal. It weighed 195 gm., was encapsulated, nodular and retained the shape of the adrenal. Microscopically it showed a typical cortical carcinoma of the adrenal. Postoperative radiotherapy was given. Within 9 months there was an almost complete *restitutio ad integrum.* A report of this case at this stage of recovery was made by Dr. Joseph Tenenbaum (*J. Urol.*, 1939, 42, 277–287). In the 14th month, however, there began a recurrence and recrudescence of all previous symptoms. In addition, there were hyperglycemia, glycosuria, hypercholesterolemia and marked hypertension. The urine was negative for estrin and prolan. Pyodermia developed. In the 18th month after operation the patient died.

Autopsy showed a recurrent tumor at the operative site and widespread metastases in the right kidney, liver, pancreas and lungs. The histological picture was, in the main, similar to the original tumor. Ponceau-fuchsin stain of the tumor by the Broster-Vines method (Broster, L. R., Vines, H. W. C., *et al.* The Adrenal Cortex Intersexuality. Chapman & Hall, Ltd., London, 1938) was negative. A most interesting finding was a miliary papillary adenoma of the anterior lobe of the pituitary, which is probably related to the basophil variety, and some changes in the basophils.

The case illustrates the following features: (1) From the gross and microscopical appearances, the impression was gained that the carcinoma probably developed on the basis of a preëxisting adenoma. (2) Unlike most other "functioning" tumors, it is the adrenal *carcinoma,* rather than the adenoma, which most frequently exhibits functional activity. (3) The association here with an adenoma of the pituitary, coupled with some changes in the basophils, is a finding which requires further elucidation in the present state of confusion between the corticoadrenal syndrome and pituitary basophilism.

ADRENAL CORTICAL NEOPLASMS: ADENOMA AND CARCINOMA ASSOCIATED WITH THE CUSHING SYNDROME. Eleanor M. Humphreys, Chicago, Ill.

Abstract. This study permitted comparisons between early and late phases of the Cushing syndrome. The patients, two women aged 47 and 29 years, had tumors

of full-fledged granulosa cell tumor tissue, which is easy if mature tissue is present, or we have to make the female character of immature parts probable by hormone determination of the tumor tissue or by the effect of these female hormones on the uterus of the patient. Among the dozen cases that Plate reviewed, only his own case, one of my cases (no. 41) and the case of Frankl (published without illustrations) correspond to Robert Meyer's definition; all other cases are either arrhenoblastomas with immature areas (for instance, the case of Eerland and Vos), or atypical granulosa cell tumors (as the case of Amati). In addition to these three cases and Black's case, a fifth case was discovered, but not yet published, by Dr. Christopoulos of Detroit. One of the cases published by M. R. Robinson (*Surg., Gynec. & Obst.*, 1930, 51, 321, case 1) is suggestive as belonging to this group: a woman, 30 years old, who, during her first pregnancy, terminated in the seventh month of gestation, showed signs of masculinization, such as growth of hair on the body and face and lowering of the voice. The right ovary, which was transformed into a tumor the size of a hen's egg, was removed. The description and four illustrations point to a granulosa cell tumor, but are not sufficient to prove the presence of arrhenoblastoma tissue as suggested by the history.

INTERSTITIAL CELL TUMORS OF TESTES IN MICE INDUCED WITH STILBESTROL. Michael B. Shimkin (by invitation) and Hugh G. Grady, Bethesda, Md. and Darby, Pa.

Abstract. Subcutaneous implantation of pellets of stilbestrol in cholesterol produces a marked hyperplasia of the interstitial cells of the testes in mice of strain C. In 6 to 12 months, about one-fourth of the animals develop frank testicular tumors, some of which metastasize. Testicular tumors of the interstitial cell type are also induced in mice of strains A and c^e, but not in mice of strains C57 Black, I, or C_3H. Foster nursing exerts no influence on the appearance of testicular tumors.

Slides were presented of the gross and histologic appearance of the induced testicular tumors in mice.

Discussion

(Dr. Emmerich von Haam, Columbus, O.) Do the tumors regress if the pellet of stilbestrol is removed?

(Dr. Robert C. Grauer, Pittsburgh, Pa.) I would like to ask what the age of these mice was when the pellets were implanted under the skin.

(Dr. Walter Schiller, Chicago, Ill.) I want to ask Dr. Shimkin whether the artificially produced interstitial tumors of the mouse testicle contain hormones, and if so, which hormones. I have had the opportunity to study slides of these tumors and the similarity with some of the human interstitial testicular tumors, for instance, with the case of Masson, is striking. However, the human interstitial tumors, if analyzed from the endocrine point of view, are not a homogenous entity. The majority of the cases found in adult men produce no endocrine changes in the patients. Three cases—those of Stuart, Bell and Roehlke; Rowland and Nicholson, and Sachi—provoked precocious puberty in small boys of 5, 6 and 9½ years. It fits into the conception of a tumor developing from cells which produce male hormones, that these growths provoke precocious puberty in small boys, analogous to the frequently observed precocious puberty with granulosa cell tumors in small girls. In an adult male organism the male hormone cannot provoke any endocrine changes. But there is a group of three cases, those of Hunt and Budd, of Huffman and of Monaschkin, in which interstitial tumors of the testicle produced demasculinization and effeminization of the patient with marked gynecomastia, twice in adults, once in a small boy. This does not fit in the conception of a tumor developing from the endocrine elements of the male gonad. The tumor of Huffman with its abundance of connective tissue and marked tendency for

GYNANDROBLASTOMA OF THE OVARY. William C. Black, Denver, Colo.

Abstract. An ovarian tumor was removed surgically from a white woman, 24 years old, who exhibited hirsutism with male voice and beard and hypertrophy of the clitoris, these changes having developed during the previous 5 years. Menstruation, although varying in the amount of flow, had always been regular with 28-day intervals until the last 4 months before hospitalization. Since then she had flowed for 5 to 7 days about every 2 weeks. Normal menstruation followed operation. She is well at present, 2½ years later, but there have been only slight changes in the hirsutism and voice, and no change in the clitoris. Histologic examination of the ovarian tumor disclosed a heterogeneous structure with extensive necrosis. In part the form and arrangement of tumor cells corresponded to that of the sarcomatoid form of granulosa cell tumor, but other portions consisted of imperfect tubules with isolated cells and groups of cells containing much lipoid material and resembling the interstitial cells of the testis. The tumor lay in the medulla of the ovary. Beneath the tunica albuginea, which formed the tumor capsule, there were marginally located large tubular glands with tall columnar epithelium including numerous mucus-secreting goblet cells.

I believe that this tumor should be classified as a gynandroblastoma, a term suggested by Robert Meyer (*Beitr. z. Path. Anat. u. z. allg. Path.*, 1930, 84, 485–520). A similar case, with a review of the literature, has been reported by W. P. Plate (*J. Obst. & Gynaec. Brit. Emp.*, 1938, 45, 254–257).

Discussion

(Dr. Walter Schiller, Chicago, Ill.) The valuable presentation of Dr. Black deserves special interest, since among the rare ovarian tumors the gynandroblastoma is one of the rarest. The term gynandroblastoma was coined by Robert Meyer in 1930, to signify an ovarian tumor composed of granulosa cell tumor tissue and of arrhenoblastoma tissue developing from a mesenchyme of indifferent cells which, when developing in a tumor, differentiate partly in the male and partly in the female line. Thus a tumor originates which in the field of neoplasms corresponds to the hermaphroditic gonad. Similarly as the hermaphroditic gonad is composed of male and female normal parenchyma, the gynandroblastoma is composed of male and female tumor tissue. Robert Meyer only suggested and anticipated this entity, but found no sufficient evidence to classify his own cases in the new group. Since the appearance of his paper, only a few cases have been published. Plate, in 1938, published 1 case and reviewed 12 cases from the literature, but of these cases only a small minority comply with the definition of Robert Meyer. It is not difficult to identify the male part of questionable tumors, since in a very early phase of differentiation, the arrhenoblastoma already shows the characteristic polyhedral Leydig cells which surround the trabeculae, long before they become canalized. The female part, the granulosa cell tumor component, offers much greater difficulties for diagnosis. It is a well known fact that in the first immature phases granulosa cell tumor and arrhenoblastoma cannot be differentiated from each other. They both present a very cellular mesenchyme which duplicates the earliest embryonic phases of the gonad and looks like a very cellular fibroma. It is sometimes called sarcomatoid, although it is immature only, and by no means malignant. Similarly as, during the first few weeks of fetal life, ovary and testicle cannot be differentiated from each other, it is also impossible to distinguish the initial immature phases of the corresponding tumors from each other. Only by adjacent parts of high maturity, can immature parts be identified as granulosa cell tumor or as arrhenoblastoma. It is an error that can easily be made, but should be avoided, to diagnose the immature part of an arrhenoblastoma as granulosa cell tumor and to classify the tumor as gynandroblastoma. To make a safe diagnosis of the female component we either have to prove the presence

The implications in these cases are that we are dealing with an obscure progressive disturbance of bone which has its inception in interference with those elements that govern proper somatic development. The cutaneous and osseous disturbance would ordinarily have its origin in an alteration in the function of the pituitary gland or possibly of the hypothalamus. The etiological factors are uncertain. Recognition of these cases is important in order that they may be differentiated from those amenable to cure through surgical removal of a parathyroid tumor.

Discussion

(Dr. M. Pinson Neal, Columbia, Mo.) May I ask the essayist if there was a disturbance in the hematological picture in these cases?

(Dr. Grauer) Do you mean in the blood counts? If so, there was none.

TRANSPLANTATION OF CHROMOPHOBE ADENOMA-LIKE LESIONS OF THE RAT HYPOPHYSIS. John A. Saxton, Jr., New York, N. Y.

Abstract. Chromophobe adenoma-like lesions of the hypophysis occur frequently at an advanced age in albino rats of the Yale strain. Homologous intra-ocular transplants were made with adenoma-like tissue from two spontaneous lesions, and tissue from one of these is now in the third serial intra-ocular generation. Growth of the transplants is extremely slow, and it has required from 12 to 19 months for the transplants to reach a size suitable for serial transfer. There is usually a latent period of several months before growth can be observed, although the transplants appear to be vascularized within a few weeks. The growth rate has not been accelerated in the second serial transfer, and the morphology has not changed. Although the adenoma-like lesions develop spontaneously only at an advanced age, the age of the recipient has been found not to be a factor either in percentage of successful transplants or in the rate of growth of transplants.

It is concluded that chromophobe adenoma-like lesions of the rat hypophysis behave on transplantation like neoplasms in that their growth rate, while characteristically slow, is independent of the growth rate of tissues of the recipients and in that the ability of transplants to grow is independent of factors associated with aging of the recipient.

Discussion

(Dr. J. M. Ravid, New York, N. Y.) I should like to ask Dr. Saxton whether he studied the relative proportions of the chromophilic and chromophobic cells in the remaining part of the pituitary; also whether he can tell us something about the growth of implanted normal pituitary tissue in the eye.

(Dr. Saxton) The relative proportions of chromophilic and chromophobic cells in other parts of the pituitary were not studied. The general impression was that there was no conspicuous change. As for the growth of normal pituitary tissue in the eye, I believe most studies have shown that normal tissue may persist under these conditions, but that it will not grow, and probably could not be carried through more than one generation.

FUNCTIONING TUMORS OF ENDOCRINE GLANDS. Howard T. Karsner,* Cleveland, O.

Abstract. Certain of the tumors which arise in ductless glands are associated with other bodily changes which can only be explained by the production of hormones by the tumors. The distinction between hyperplasia and neoplasia presents the same difficulties as in the exocrine glands. What are clearly hyperplasias may have the same hormonal activities as the tumors and this feature is of no aid in making the distinction.

* By invitation of the Council.

calcification differs morphologically from the ordinary type. The conception may be raised whether these three effeminizing tumors may be hypernephromas. The effeminizing potency of some adrenal hypernephromas in adult man is well known and remnants of adrenal cortex have been found in the hilum of the testicle in a few cases in the human and are not too rare in certain animals, as in the rabbit. Hormone determinations in artificially produced interstitial tumors in animals and in clinically diagnosed tumors in humans may help to clarify this problem.

(Dr. Shimkin) I do not know whether these tumors regress if the pellet is removed. The pellet was permitted to remain in place in all these animals. In no case did regression occur under these circumstances. The pellets were usually implanted when the mice were 2 to 3 months of age. However, we have implanted them when the animals were barely mature (28 days) to 8 months of age, without any definite difference in effect.

Concerning the hormones, if any, secreted or stimulated by the tumors, I do not know. We were trying to get a sufficient amount of tumor tissue from the transplants for the studies since one cannot get enough from the original tumors. Gardner believes these tumors do have a hormonal effect, but our evidence is inconclusive.

THE INFLUENCE OF THE THYMUS GLAND ON NEUROMUSCULAR FUNCTION IN MYASTHENIA GRAVIS. A. M. Harvey, J. A. Lilienthal, Jr., and S. A. Talbot (*by invitation*), Nashville, Tenn.

Abstract will appear in the Proceedings of the American Society for Clinical Investigation, published in *The Journal of Clinical Investigation*. The full paper will appear in the September, 1942, issue of that journal.

Discussion

(Dr. Seaton Sailer, Cincinnati, O.) What were the histological changes in the thymus tissue removed?

(Dr. Harvey) None of the patients had a localized tumor of the thymus; there was a generalized hyperplasia of the small round cells of the thymus gland. There were no changes in the epithelial cells in these cases.

(Dr. William H. Harris, New Orleans, La.) I should like to ask if there was any enlargement of other discernible lymphoid structures.

(Dr. Harvey) In none of the cases was there any palpable enlargement of the lymph glands.

FIBROUS OSTEODYSTROPHY ASSOCIATED WITH PIGMENTATION OF THE SKIN. Robert C. Grauer, Pittsburgh, Pa.

Abstract. Differentiation of fibrous osteodystrophy in which there is an absence of demonstrable parathyroid overactivity must be made from those forms in which there is a disturbance in the parathyroid gland. A specific entity in which the osseous disturbance is associated with congenital pigmentation of the skin was described. Two cases, one in an adult female who had been studied over a period of 20 years, and the other in a male child, were presented. Complete studies, which included determinations of calcium balance, hormonal assays, repeated roentgenological examinations and multiple biopsies of various portions of the skeleton, were performed. Subsequent parathyroid exploratory operation was done. The patients are often indistinguishable from those cases showing parathyroid adenomata. The chief characteristics of the condition are a disseminated cystic osteodystrophy; areas of melanotic cutaneous pigmentation in the regions of the greatest bony disturbance; positive calcium balance and evidence of alteration in bone growth as influenced by the endocrins.

showed a large ulcerating carcinoma in the region of the pylorus and there was a small rounded meningioma attached to the dura mater over the left frontal area 3 cm. from the middle line. There was atheromatous change in the aorta and the coronaries, and the arteries generally, especially those of the lower limbs, were extensively calcified. In regard to the endocrine glands, the adrenals were large but normal and the pituitary was flattened, as was also the brain, by a projection upwards of the base of the skull; the thyroid was small and showed nothing of note microscopically; the parathyroids were dissected out and found to be normal.

Microscopically the bony changes were in conformity with those usually found in Paget's disease, the only unusual feature being well marked calcification of the media of the arterioles in the substance of the bone itself.

Discussion

(Dr. W. E. B. Hall, Port Huron, Mich.) I should like to ask Dr. Miller if, in the examination of the bones, he found any areas showing giant cell formation, and also if he found any areas in which considerable hyperplasia of the osteogenic elements was present, such as we might expect to find preceding the formation of forms of osteogenic sarcoma.

(Dr. Miller) There is one point which I should have mentioned: tumors are extremely frequent in these cases, and this patient had a carcinoma of the stomach and a meningioma—a typical psammoma of the dura mater—but she had no tumors of the giant cell type, and nothing to suggest osteogenic sarcoma.

RELATIONSHIP OF NEUROFIBROMATOSIS OF THE SKELETON TO PSEUDO-ARTHROSIS. William T. Green and Nathan Rudo (by invitation), Boston, Mass. Presented by Ruell A. Sloan.

Abstract. Although pseudo-arthrosis and "congenital bowleg" have been described as skeletal changes which may occur with neurofibromatosis, the mechanism by which pseudo-arthrosis arises has not been explained, nor are we able to find a pathologic description of tissue from the area of pseudo-arthrosis.

A patient, a white female, 7 years of age, was first seen complaining of a tender mass in her right arm of 8 months' duration, and a bowing of the right leg which had been present since birth. Pertinent physical and roentgenographic findings at this time included café-au-lait spots on the skin, a tumor mass of the right arm which was demonstrated microscopically to be a plexiform neuroma, an antero-lateral bowing of the tibia with increased density of bone and a pseudo-arthrosis of the fibula. In the patient's subsequent course, a localized tumor developed in the tibia, and two pathologic fractures occurred in this bone. One of these has not united despite surgical intervention.

Histologic examination of tissue from the pseudo-arthrosis of the fibula, from the tumor defect in the tibia and from the pathologic fractures of the tibia all revealed typical neurofibroma. In this case, the pseudo-arthrosis seemed to be associated directly with intra-osseous neurofibromata which had caused pathologic fractures of the tibia and fibula and retarded or prevented bony union.

In all cases of so-called congenital pseudo-arthrosis, the possibility that neurofibroma is the etiologic factor must be entertained. Tissue removed from the area of the pseudo-arthrosis at operation should be scrutinized carefully in an attempt to demonstrate or exclude the possibility of neurofibroma, realizing that superficial examination may suggest the diagnosis of "scar tissue." It should be emphasized that it may be difficult to differentiate neurofibroma from fibrous tissue of other origins. If neurofibroma is present, incomplete excision of the tissue may lead to recurrence and be a factor in non-union.

The tumors may be benign or malignant, but the microscopic criteria usually characteristic in tumors of exocrine glands may be misleading in the tumors of endocrine glands. Thus tumors which are obviously benign may show marked cellular pleomorphism, alteration of polarity and many mitoses. The malignant forms often show these changes, but the alterations may be inconspicuous in the original tumor and its metastases. A diagnosis of malignancy rests on the establishment of invasion, or metastasis, or both. Recurrence, as a sign of malignancy, is undependable, because aberrant rests of glands or their anlagen may give rise to new tumors independently of the original neoplasm.

Regardless of the embryonal origin of the glands, the terms adenoma and carcinoma are usually applied and have obtained good usage. Nevertheless, certain tumors of the ovary, those of the adrenal cortex and perhaps of other situations, are ultimately derived from mesoblast.

The association of special functional changes with tinctorial and other properties of the neoplastic cells does not seem to be wholly justified. There are certain variations from the expected in acidophilic and basophilic lesions of the pituitary. The functioning islet cell tumors of the pancreas have not been shown to possess any unusual numbers of beta cells. There is no clearly established relationship between any particular cell of the adrenal cortex and the changes in secondary sex characters. The lipid-containing cells sometimes seen in arrhenoblastomas of the ovary are not necessarily homologues of the interstitial cells of the testis.

It is usually assumed that hormones are produced because of the somatic effects of the tumors. In only a few of those associated with gonads and adrenals has there been any satisfactory assay of hormones and such assays are practically limited to the steroidal sex hormones. The influence of intermediate products and metabolites is not yet fully established, nor is the influence of quantities and proportions altogether clear. Further studies should be made at every opportunity and may aid not only in explaining the activity of the tumors but also in the final identification of some of those whose nature is not yet established.

A Case of Paget's Disease of Bone with Autopsy Report. James Miller, Kingston, Ontario.

Abstract. The patient, E. S., was born in the south of England in 1870. She was a widow and previous to marriage worked as a weaver. She was a member of a family of six; four girls and two boys. There is no record that either of the parents suffered from bone disease, but all four sisters have developed symptoms suggestive of Paget's disease and one, who is resident in Canada, has distortion of most of the bones of the body and has had repeated spontaneous fractures. There are no precise data of the onset of the disease in the patient but it was apparently well marked in 1928. She was admitted to Rockwood Mental Hospital in 1940 and died in March, 1942. The diagnosis in her case was senile psychosis with simple deterioration. Clinical pathological observations carried out during hospitalization were as follows: serum calcium, 10.2 mg.; blood urea, 24 mg.; hemoglobin, 60 per cent; red blood cells, 2,580,000; leukocytes, 5,760. The Wassermann test was negative.

The relevant autopsy findings were as follows: The calvarium was enormously enlarged, measuring 63.5 cm. in circumference. When removed, it weighed 1740 gm. It varied in thickness from 1 to 3 cm. The distinction between diploë and outer and inner tables had disappeared, the whole bone being transformed into soft osteoid material red in color and easily cut with the saw, and some parts with the knife. The spine showed well marked kyphosis in the dorsal region. The long bones, radius, ulna, femur and tibia, were bent, thickened and softened, and the marrow of the shafts was red and partially occupied by soft bone. The stomach

The character of the lesions in the heart, kidneys and other organs in these cases gives further support to the view that rheumatoid arthritis is in all probability an infectious disease. The nature of this infection is not clear from this study, but the high incidence of rheumatic cardiac lesions in this series is suggestive of a relationship between chronic infectious arthritis and rheumatic fever.

Discussion

(Dr. Jacob Werne, New York, N. Y.) I am very much interested in the lesions, other than those rheumatic in character, which Dr. Baggenstoss described. I have encountered glomerular changes of the type described, and foci of cellular infiltration in visceral sections in my series of infants dying unexpectedly of fulminating infection. May they not be considered as manifestations of the acute infections which brought these cases to autopsy, rather than as lesions that are specifically part of the chronic infectious arthritic disease? To phrase the matter differently, I wonder whether these same lesions would be found in cases dying of intercurrent violence rather than of their infectious arthritis.

(Dr. Baggenstoss) Of course it is impossible to state in every instance whether the lesions described were the result of a terminal infection or whether they were an integral part of the disease known as rheumatoid arthritis. I think there are two things to bear in mind. Almost all of these patients came to the clinic with an active rheumatoid arthritis. The joint disease was not quiescent; the patients had an active arthritis and came for the treatment of this condition. Another thing to bear in mind from Dr. Bell's study on glomerulitis is that in patients dying with lobar pneumonia, only about 18 per cent have a glomerulitis and yet in the present group of cases 63 per cent had glomerulitis. Terminal pulmonary infections would not account for such a high incidence.

CHEMICAL ISOLATION WITH PRESERVATION OF STRUCTURE AND FUNCTION OF ELASTIC TISSUE OF THE HUMAN AORTA. George Hass, New York, N. Y.

Abstract. This report was a presentation of a method for dissolving all components of the fresh human aorta except elastic tissue. The elastic network with all preexisting structural continuities and staining properties are preserved in such perfect state that a pure tissue becomes available for correlative physical, morphological and chemical studies. The pure tissue is more elastic in the common sense than the aorta from which it is obtained and displays various other properties which cannot be estimated by studies of the intact aorta.

A FATAL DISEASE OF MIDDLE-AGED MICE WITH MYOCARDITIS ASSOCIATED WITH HEMORRHAGE IN THE PLEURAL CAVITY.* D. Murray Angevine and Jacob Furth, Wilmington, Del. and New York, N. Y.

Abstract. A disease complex hitherto not described is occurring in small numbers of all stocks of normal mice in an animal colony comprised of from 5,000 to 7,000 mice per annum. It is found in about 1.2 per cent of all mice dying spontaneously and is characterized by myocarditis with sudden death, usually from hemorrhage into the pleural cavity. The hemorrhage is due to rupture near the origin of the great vessels. Gross or microscopic testicular hemorrhage was usually present. Hyaline degeneration of blood vessels in the lungs and testes was frequently observed. The disease affects male mice with rare exception. It is most common between 10 and 19 months with a peak at 14 months. Healthy mice are apparently affected and they appear normal until death occurs.

Transmission experiments to normal mice have been unsuccessful and cultures failed to yield an organism that could be regarded as causing the disease. Epidemiological observations likewise did not indicate an infectious disease.

* Accepted for publication in the *American Journal of Pathology.*

Discussion

(Dr. Edward A. Gall, Cincinnati, O.) I wonder if the authors have any explanation for the peculiar sigmoid deformity of the tibia.

(Dr. Sloan) The cause of the bowing of the tibia is not readily apparent. We have seen several other patients with a bowing of similar character in association with neurofibromatosis. The discovery of tumor tissue in the shaft of the bone might lead one to speculate as to the possibility of the presence of neurofibroma at the zone of enchondral ossification, causing inequality of the rate of bone growth and consequent deformity. Under such circumstances, with progression of the growth zone away from the tumor, the normal rate of growth might be re-established. No evidence to support this theory could be determined, as roentgenographically no definite defects in the tibia were demonstrated before the child was admitted to the hospital at the age of 7 years.

(Dr. Robert C. Grauer, Pittsburgh, Pa.) Was the rest of the skeleton x-rayed in order to determine whether any other defects were present by roentgenologic evidence?

(Dr. Sloan) Skeletal survey showed the remainder of the skeleton to be free of lesions.

VISCERAL LESIONS ASSOCIATED WITH CHRONIC INFECTIOUS (RHEUMATOID) ARTHRITIS. A. H. Baggenstoss and (by invitation) E. F. Rosenberg, Rochester, Minn.

Abstract. Many investigators feel that rheumatoid arthritis is a generalized systemic disease and that arthritis is merely one of its manifestations. The present study was undertaken primarily to determine the nature of the anatomic changes which occur in the viscera in rheumatoid arthritis. It was based on a study of the necropsies in 30 cases of chronic infectious (rheumatoid) arthritis. There were 17 males and 13 females. The mean age of these patients at the time of death was 38 years. The youngest patient was 9 years and the oldest 81 years of age. Only 5 patients were 60 years old or over.

Cardiac lesions were present in 24 cases. Lesions indistinguishable from those of rheumatic fever were observed in 16 cases, or 53 per cent. Non-rheumatic lesions were present in 8 cases (27 per cent). In 9 of the 16 cases which had rheumatic cardiac lesions the heart disease was judged to be an important factor in causing death. The lungs were frequently the site of infectious processes in patients with rheumatoid arthritis but it is difficult to consider these as being anything more than coincidental. Many of the pulmonary infections were, in fact, terminal. A slight degree of splenic enlargement was common. The mean weight was 250 gm. The spleen weighed over 300 gm. in 9 cases. In most of these cases the enlargement was found, histologically, to be on the basis of either chronic passive congestion or reticulo-endothelial hyperplasia. Amyloid disease occurred in 2 cases. Chronic passive congestion of the liver was present in 18 cases, fatty change in 7 cases. Atrophy of the hepatic parenchyma was present in practically all of the cases which had passive congestion. Actual necrosis of the hepatic cells occurred in 4 cases. Inflammatory lesion occurred in the intestinal tract in 4 cases, in the pancreas in 3 cases, in the prostate in 2 cases and in the adrenal gland in 2 cases. Subacute suppurative interstitial nephritis occurred in 3 cases, and non-suppurative pyelonephritis occurred in 1 case. Proliferation of the endothelial cells of the glomerular capillaries (glomerulitis) was present in 19 cases (63 per cent). Nine of the 19 cases (47 per cent) with glomerulitis had evidence of either active or healed endocarditis. It may be that in these cases the renal lesion was a result of the endocarditis, but we feel that it is more reasonable to attribute both cardiac and renal lesions to the same underlying disease.

eosinophilic promyelocytes and myelocytes, only the last blood examination a short time before death showed a low percentage of eosinophilic metamyelocytes and polymorphonuclears. Slides of the liver gave evidence of a gradual transformation of Kupffer cells into eosinophilic myelocytes by the development of eosinophilic granules and retraction of the stellate projections of protoplasm. After this metaplastic change, the cells entered the blood of the sinusoids as eosinophilic granulocytes.

UNRELIABILITY OF BLOOD FINDINGS AS CRITERIA OF SEVERITY OF SHOCK IN RABBITS. Milton D. Bosse (by invitation) and Paul Gross, Pittsburgh, Pa.

Abstract. Seventy rabbits were burned by partial immersion in hot water under ether anesthesia. Although in the majority of animals varying degrees of hemoconcentration and hypoproteinemia were demonstrable in addition to clinical manifestations of shock, a number of notable exceptions were encountered. It was found that in a number of animals which died of shock, the hemoconcentration was quickly followed by hemodilution, and in several the picture of shock was associated with hemodilution from the onset. The degree of hemoconcentration was not an accurate indication of the degree of shock because many of the animals that died exhibited a relatively mild hemoconcentration. In some rabbits dying of shock the blood protein had returned to the normal level, or above, at the time of death. These exceptions do not conform to results obtained with other animals and cannot be explained at the present time.

Discussion

(Dr. Virgil H. Moon, Philadelphia, Pa.) I am interested in this investigation, and I can support the essayists' findings of hypoproteinemia after burns and incident to shock from other causes. However, I should like to ask the essayists if they have used other animals than rabbits in any of their experiments. We have made various experiments on different types of animals and have found results more variable in rabbits than in any other species. Some of our early experiments on shock were made on rabbits. These showed more individual variation than did other animals; accordingly, our subsequent experiments were made on dogs in preference to rabbits. Did you make your experiments on any other animals?

(Dr. Bosse) These experiments are reported because, if the present theories of shock are correct, they should apply to all animals which manifest shock. We worked only with the rabbit in the experiments and the results obtained do not conform.

(Dr. Moon) That coincides with our experience. I should like to ask whether the hemoconcentration was observed by means of the determination of the specific gravity of the blood, by hemoglobin and red cell counts, or by the hematocrit.

(Dr. Bosse) The plasma protein was determined by the micro-Kjeldahl method. The hemoconcentration was determined by the red blood cell count, although in most cases hematocrit readings were also made and the changes corresponded very closely to those in the red blood cell counts.

(Dr. Moon) We have come to the conclusion that these determinations are best made by the red blood cell count. I think this is more accurate for determining hemoconcentration than is the specific gravity of the blood.

HEMATIC AND ORGANIC CHANGES AFTER INTRAVENOUS ADMINISTRATION OF PECTIN SOLUTIONS. W. C. Hueper, New York, N. Y.

Abstract. Rabbits and dogs were injected with neutralized 1 and 2 per cent colloidal pectin solutions over periods of several months. The immediate hematic effects consist in a colloidoclastic leukopenia lasting for several hours and a hastened

Discussion

(Dr. Jacob Werne, New York, N. Y.) I would like to ask Dr. Angevine whether he did any bacterial stains on his visceral sections. I would also ask why he considered the *Staphylococcus aureus* as a contamination. In my human material I have occasionally found acute myocarditis in persons dying of fulminating infection. In several instances that I recall, *Staph. aureus,* probably entering through the upper respiratory tract, was the offending organism.

(Dr. Stuart Lippincott, Bethesda, Md.) Occasionally we see myocardial lesions in strains of mice such as the C_3H strain, and usually they are preceded by degeneration, followed by calcification, but there is no other lesion in the body. I wonder whether in the old mice of this colony you ever see calcification.

(Dr. Angevine) In answer to Dr. Werne's question, we made many bacterial stains on the viscera of these animals, and found nothing significant. If I said *Staph. aureus,* it was a mistake, for I meant *Staphylococcus albus.*

The majority of mice of this colony died before 12 months, so that we did not have many animals reaching old age. However, calcification was not observed in the hearts of the older mice examined.

LOCAL ORIGIN IN HUMAN EXTRAMEDULLARY MYELOPOIESIS. Walter Schiller, Chicago, Ill.

Abstract. Two explanations have to be considered for the development of extramedullary myelopoiesis. The first is the assumption of local origin. This theory meets with greater probability when the organs in question are those normally concerned with hematopoiesis in fetal life, as liver and spleen. With anemias the presence of erythropoietic tissue in these organs may be interpreted as a compensatory revival of a prior function which physiologically is not carried on in extra-uterine life. A subgroup of the theory of local myelopoiesis which finds good support in many animal experiments concerns the transformation of vascular endothelium (Dieckmann), of perivascular adventitial cells (Bloom, Herzog), or of undifferentiated mesenchymatous elements mostly located in the vicinity of blood vessels (Jaffé, Lang), into undifferentiated lymphoid cells or hemocytoblasts, after irritation or stimulation by injection of dead bacteria, as proteus or coli; of poisons, as saprotoxin, or pyrogallol, or after inoculation with transplantable tumors. The second theory traces the extramedullary foci of myeloic tissue back to hematogenous immigration of immature bone marrow cells which enter the circulation in the bone marrow. This process, which is analogous to the metastasizing of malignant tumor cells by the blood stream, has been called colonization by some authors, a term coined to use instead of "metastasizing."

Local origin is much the more difficult to prove. Two ways are open. The first is to find evidence of local transformation of mesenchymatous cells, particularly of cells of the reticulo-endothelial system into immature blood cells; that is, metaplasia *in situ.* The second way is to find a case which shows abundance of the immature blood elements in the bone marrow and in well developed extramedullary foci but with the circulating blood free from such cells. Quantitative differences between the incidence of immature blood cells in the blood and in the tissue do not prove much, since even a few intravascular cells may give origin to extensive metastases as is observed in malignant tumors. Only qualitative differences are convincing and particularly the absence of the immature elements in the blood, which are present in the tissues. After unsuccessful examination of numerous cases of acute and chronic lymphatic and myelogenous leukemia, a colored woman, 46 years old, with chronic myelogenous leukemia was found who expired when the neutrophilic leukemia was on the verge of changing into an eosinophilic one. Whereas the bone marrow and the liver at autopsy contained huge numbers of

During this period the parietal and chief cells of the acini appeared normal, and the neck cells were still filled with mucus. As stasis and hemorrhage increased, mantles of red blood cells became confluent. At this time many of the mucus-secreting neck cells disappeared, but some of them persisted. Simultaneously the acini began to lose their normal cellular arrangement. The lumina of acini disappeared as the margins of the cells became indistinct. Nuclei of adjacent cells became pyknotic. Shallow ulcers formed as the result of whole groups of acini becoming necrotic, together with the destruction of the overlying layer of mucous cells. In sections stained by hematoxylin and eosin the ulcers often appeared yellowish green, and this suggested bile deposition. However, with Stein's technic bilirubin was not found. With the loss of overlying necrotic tissue, the margins and bases of these ulcers became clearly defined. Inflammatory exudate was absent. The ulcers were usually limited to the outer half of the mucosa but occasionally extended down to the muscularis mucosae. It is apparent that the process did not originate with a primary local loss of continuity in the layer of mucus-secreting cells. Instead, there was a local circulatory disturbance with subsequent interference in the nutrition of all the cells of that region which resulted in necrosis of acini and the formation of superficial mucosal ulcers.

Discussion

(Dr. Carl V. Weller, Ann Arbor, Mich.) I noted with interest reference to the rather heavy eosinophilic infiltration. We were asked recently to examine the alimentary tracts of rats used by Professor Norman Maier of the Department of Psychology of the University of Michigan, in the production of neurosis and psychoneurosis in rats by thwarting conditioned reflexes. One of our internists thought those rats should have ulcers after such an experience, and we were asked to examine them. I found very heavy eosinophilic infiltration in most of them, and no active ulcers. A very small percentage showed acute erosions which might be beginning ulcers. The only explanation which I found for the eosinophilia was the presence of nematodes in considerable numbers in the intestinal tract. I mention this without any disparagement of the authors' results, but I think the presence of eosinophils should be interpreted with caution.

HISTOGENESIS AND REPAIR OF HEPATIC CIRRHOSIS IN RATS PRODUCED ON LOW PROTEIN DIETS AND PREVENTABLE WITH CHOLINE. R. D. Lillie, L. L. Ashburn and (by invitation) W. H. Sebrell, F. S. Daft and J. V. Lowry, Bethesda, Md.

Abstract. The hepatic cirrhosis of rats produced by Lillie, Daft and Sebrell on low protein, choline-deficient diets is characterized by centrolobular fatty changes, soon followed by the appearance of an acid-fast sudanophilic hyaline material, designated as ceroid, in liver cells and interstitial, subcapsular and centrolobular phagocytes. Soon after this trabeculae of ceroid phagocytes in fibrous tissue form. Choline treatment removes the fat from the liver cells and increases their size, but leaves untouched the fibrous trabeculae and ceroid phagocytes. This cirrhosis differs from any we have seen in man and from other experimental cirrhoses.

VARIATIONS IN THE DISTRIBUTION OF VITAMIN A IN THE HUMAN LIVER UNDER PATHOLOGIC CONDITIONS. Hans Popper, Chicago, Ill.

Abstract. Various pathologic conditions of the liver reveal under the fluorescence microscope characteristic variations in the amount and distribution of vitamin A. These changes (in human liver) are frequently more characteristic than those found with routine histology. Animal experiments with various diets and intoxications aided in recognizing the significance of some of the typical changes in the pathologic human liver. An inability of the damaged epithelial cells to hold or discharge

erythrocytic sedimentation lasting for more than a week. The hematic effects of repeated injections consist mainly in an accelerated sedimentation rate. Pectin is stored in the liver, spleen, kidney and bone marrow, giving rise to a foam-cellular transformation of the reticulo-endothelial cells of these organs as well as of the liver cells. Giant-cellular granulomas are found in the pulmonary arterioles. Atheromatous lesions of extensive degree and medial degeneration and calcification are elicited by pectin in the aorta and in large and small arteries. Thus, additional evidence is advanced in support of the thesis that macromolecular colloidal disturbances of the blood plasma are causally related to the development of arterial atheromatosis and organic thesaurosis.

Discussion

(Dr. Hans Popper, Chicago, Ill.) I would like to ask Dr. Hueper the following questions: First, how did the dosage of pectin he used compare with the doses Hartman and his associates used as blood substitutes in man? Second, did he use autoclaved pectin solutions as Hartman did and, if so, what results he got with them? Third, how long did the storage of the material in the organ last?

(Dr. Hueper) Our dosage cannot be compared with the dosage of the Hartman experiments, because this investigator used autoclaved pectin solutions. When a pectin solution is subjected to autoclaving, a heavy precipitate is formed, which is especially marked if the solution was neutralized before being subjected to heat. This precipitate is filtered off and thus a severe loss of pectin is sustained. In addition to this reduction in concentration of pectin, an autoclaved solution contains also a pectin of considerably decreased molecular weight. The heat degradation of pectin manifests itself in a considerable lowering of the degree of viscosity. Bryant has prepared a chart in which the depolymerization of the pectin by hydrolysis under the influence of autoclaving is demonstrated. He found that a pectin solution consisting originally of molecules having a molecular weight somewhat above 200,000 was composed, after being autoclaved for 30 minutes at 15 pounds pressure, of molecules of an average molecular weight of about 50,000. In my experiments with autoclaved pectin solution there was little retention of the degradation product in the internal organs. In some of the animals foam cells were found in the bone marrow. It is my opinion that pectin solutions are unsuitable for the treatment of shock, as they cannot be well standardized as to concentration and physicochemical properties when subjected to autoclaving and are highly unstable even when kept in the ice box.

HISTOGENESIS OF GASTRIC ULCERS IN RATS PRODUCED BY FASTING AND PARTIAL INANITION. Stuart W. Lippincott and (by invitation) Harold P. Morris, Bethesda, Md.

Abstract. The production of gastric lesions in rats by several experimental procedures has attracted the attention of many investigators during the last quarter of a century. The etiologic factors presumably involved have varied considerably. Of significant interest has been the purported relation of certain dietary deficiencies to the genesis of ulcers of the glandular portion of the stomach. Our investigations upon 14 experimental groups of rats showed that superficial ulcers of this region developed during fasting and partial inanition, but never were observed during the individual absence from the diet of fat, carbohydrate, or protein.

It was observed microscopically that a circulatory disturbance initiated the changes resulting in the formation of the mucosal ulcers of the glandular portion. The peripheral capillaries, in focal areas, became hyperemic, followed by escape of red blood cells. The spread of the red blood cells was restricted at first to the relatively small amount of loose connective tissues lying between the intact acini.

adhesion in the commissures, which is easily overlooked, and there is often fibrosis along the line of closure or the free border. In the mitral valve, as Dr. Hall described, there is thickening along the free border, sometimes accompanied by adhesions between the leaflets, as well as thickening of the chordae tendineae; vascularization of the anterior leaflet frequently occurs. There is also more or less marked roughening of the posterior wall of the left atrium. In our last 100 consecutive autopsies, gross findings were present in 30 cases. We think of these as non-deforming in the sense that they probably, and almost certainly, produced no functional lesion in the valvular orifice. We think they probably represent a healed lesion in the valves and I believe we agree with Dr. Hall in that respect. If a pathologist refuses to make a diagnosis of valvular disease unless he finds changes sufficiently marked to produce functional disturbance, such as pronounced adhesions of the commissures, or retraction of the cusps and leaflets, he will miss many cases.

We are of the opinion that the early lesion of rheumatic endocarditis may become a progressive chronic inflammation which ultimately leads to marked deformity, or that the lesion may become quiescent to remain in a healed stage for many years with no evidence of stenosis or insufficiency. That these healed lesions are the sequel of rheumatic disease is a problem deserving the careful study given by Dr. Hall. We pay little attention to the history of rheumatic fever, not merely because of the fallibility of histories but also because of the likelihood that rheumatic fever may be sub-clinical. The work of Karsner and Bayless showed the frequency of disease of the coronary arteries in rheumatic disease, but did not prove these changes to be pathognomonic. It may be that some of the valvular disease referred to, quiescent and non-deforming, may be the result of other forms of acute endocarditis, but every available clue points strongly toward rheumatic origin.

(Dr. Hall) I am grateful to Dr. Karsner for the discussion. I am especially glad that he accepts the thickening of these valves as being probably rheumatic. I did not have time to go into it as carefully as he did. I personally performed about 25 per cent of these autopsies.

(Dr. Karsner) Let me interrupt you. I did not perform any of them myself, but I saw the material from all.

(Dr. Hall) We have about 1,100 autopsies a year, and I cannot do them all myself. There were only 3 cases of the 112 studied in which a history of rheumatic fever was recorded. I might say that Karsner and Bayless was my bible in this study.

READ BY TITLE

A Transplantable Spontaneous Hepatoma in a Strain C_3H Mouse. Jesse E. Edwards and (by invitation) Albert J. Dalton and Howard B. Andervont, Bethesda, Md.

Abstract. A primary spontaneous hepatoma in a male C_3H mouse, 17 months old, of the Andervont line has been successfully transplanted for 4 generations in the subcutaneous tissues of mice belonging to the homologous strain and line. Grossly, the primary tumor was a globular, discrete, pale yellow mass 1.2 cm. in diameter. Microscopic examination showed it to consist of liver-like cells with faintly basophilic cytoplasm and well-defined cytoplasmic membranes arranged in obvious cords alternating with endothelial-lined blood sinuses of varying width. No pigment was demonstrated. The tumor was not encapsulated and compressed the adjacent hepatic tissue. The neoplasm could be distinguished from liver by the relatively large cells with basophilic cytoplasm, by the absence of a definite lobular pattern and the absence of bile ducts, except for a few distributed haphazardly in peripheral areas. There were no metastases.

vitamin A; a block between Kupffer and epithelial cells in its transmission, and an inability of the Kupffer cells to absorb vitamin A from the blood or to split carotene were demonstrated. Conclusions as to vitamin A therapy were drawn.

THE INCIDENCE OF RHEUMATIC STIGMAS IN NON-RHEUMATIC HEARTS. Ernest M. Hall and (by invitation) Lucille R. Anderson, Los Angeles, Calif.

Abstract. In a previous study an attempt to find normal hearts that showed no rheumatic stigmas when examined according to the standard method of Gross, Antopol and Sacks was unsuccessful. It seemed worth while to investigate the matter further. The authors have studied 124 hearts; 12 of these revealed evident lesions of rheumatic or bacterial endocarditis and were used as controls. The remaining 112 hearts were free of evident gross lesions of the valves although many revealed minimal thickening of valve edges. Some of the hearts in the larger group were sectioned according to the standard methods. In the majority blocks were sectioned from only two areas: (1) through the posterior papillary muscle; (2) through the base of the left ventricle including a portion of the posterior mitral leaflet.

The more common stigmas which are usually considered indicative of rheumatic infection were employed; *viz.,* rheumatic arteritis, fibrinoid necrosis and elastic tissue alterations, Aschoff bodies, perivascular fibrinoid swelling or fibrillary scarring, increase in cardiac histiocytes in the region of the vessels and in the interstitial tissue. Sixty-eight, or 60.8 per cent, of the 112 hearts exhibited abundant rheumatic stigmas and were considered "positive" for rheumatic infection. Thirty-six hearts (32.1 per cent) were moderately involved and were called "probable rheumatic infections." Only 8, or 7.1 per cent, were considered doubtful. Aschoff bodies were found in 33 hearts (29.5 per cent) while Aschoff-like nodules consisting of cardiac histiocytes were seen in 34 cases, or 30.3 per cent. Among the 12 control cases there was a history of rheumatic fever in 9 cases. Aschoff bodies were found in 9 of the 12 cases. Of this control group rheumatic stigmas were abundant in 11 and moderate in 1.

Do the results of this study signify a practically universal rheumatic infection in much the same sense as tuberculosis is essentially universal? If so, most persons are relatively immune just as they are to minute quantities of tubercle bacilli. The etiologic agent in rheumatic fever is unknown but much evidence points to the hemolytic streptococci as either directly or indirectly of importance. The rheumatic stigmas found in clinically and anatomically non-rheumatic hearts may represent only hyperergic reactions to recurrent sensitizations with the proteins of hemolytic streptococci. These may occur with attacks of upper respiratory infections. It makes no difference, however, whether the etiologic agent is a streptococcus, a virus, or some other organism, since the principle remains the same.

Discussion

(Dr. Howard T. Karsner, Cleveland, O.) In Cabot's book, "Facts About the Heart," he described incidentally, rather than as a major part of the work, what he calls non-deforming chronic endocarditis. He did not, however, definitely attribute this lesion to rheumatic disease. During the course of the past 2 years we have been watching for non-deforming chronic disease of the valves and our data are in general agreement with the observations of Dr. Hall. Valvular disease of the heart occurs much more frequently than would appear on casual examination. A striking feature is the thickening along the edge of the anterior leaflet of the tricuspid valve, often accompanied by some fusion of the chordae tendineae. The pulmonic cusps are likely to show transverse striae of fibrosis and often a slight deformity along the free border. The aortic cusps often show slight sub-marginal

apparently is caused by differential chemical structures involved. The higher molecular weight substances—globulins—precipitate in lower dilutions. Those of smaller molecular weight—albumins—combine in higher dilutions.

REDUCTION OF TUBERCULIN REACTIVITY FOLLOWING INHALATION OF FUMES FROM BOILING BACILLI. Sol Roy Rosenthal, Chicago, Ill.

Abstract. In the Tice Laboratory, in which the BCG vaccine (Bacillus of Calmette and Guerin, an avirulent tubercle bacillus) is prepared and administered, it was noted that certain members of the staff would develop periodically symptoms varying from malaise to chills, fever (up to 104° F.), tickling sensation in the posterior pharynx, cough, vomiting, headache and backache. After several months, such responses lessened and finally abated. The possibility that the above symptoms might be related to a generalized tuberculin reaction was suspected for the first time when a new member of the staff who reacted to 0.00001 mg. of O.T. developed a similar syndrome. In tracing the source, it was discovered that the periodicity of the responses was related to the boiling of cultures or vaccine. It was inferred that the vapors might be the offender and in order to test this assumption, animal experimentation was resorted to.

The method employed was to allow fumes from a boiling vessel to enter a sealed box in which guinea pigs were placed. Visible vapors should permeate the enclosure at all times. The time of exposure for animals that reacted to tuberculin (following intraperitoneal injection with BCG) was 1 minute the first day, increasing by 1 minute daily the first week, 2 minutes the second week, and 3 minutes the third week until 60 minutes was reached. The period of exposure to the vapors at one time was never over 15 minutes (to prevent suffocation). Tuberculin testing was performed at regular intervals using old tuberculin and purified protein derivative. In numerous experiments using more than 100 animals, the following observations were made: The cutaneous response to tuberculin could be appreciably reduced in guinea pigs, made reactive by the injection of avirulent tubercle bacilli, following daily exposures to: (1) fumes of boiling suspension of tubercle bacilli; (2) sprays of heat-killed bacilli, and (3) fumes of boiling suspension of tubercle bacilli passed through a fritted glass filter (to trap the bacilli when they issued with the vapors).

THE PATHOGENESIS OF THE LESION ASSOCIATED WITH PANCREATIC ACHYLIA. Sidney Farber, Boston, Mass.

Abstract. A lesion of the pancreas characterized by dilatation of the ducts, inspissation of secretion and connective tissue replacement of atrophied acinar structures is found in constant association with two apparently distinct, clinical pictures in infants: (1) The pancreatic fibrosis variant of the celiac syndrome, and (2) meconium ileus. Evidence is presented to show that the time at which the pancreatic lesion occurs determines whether the clinical picture will be that of the celiac syndrome or of meconium ileus, and that pancreatic achylia secondary to interference with the liberation, formation, or passage into the duodenum of pancreatic enzymes is the defect of fundamental importance in both clinical pictures. Although pancreatic achylia may be produced in these patients by congenital atresia or severe stenosis of the pancreatic ducts, it is unassociated in the vast majority of instances in our experience with any such gross anatomical interference to the outflow of pancreatic juice. These studies do not permit the conclusion that either vitamin A-deficiency disease or a filtrable virus infection is of primary importance in the production of this pancreatic lesion. Although a defect in the absorption of vitamin A is a feature of the pancreatic variant of the celiac syndrome, this pancreatic lesion occurs in the absence of evidence of vitamin A-deficiency disease and persists after the disturbance in absorption of vitamin A has been corrected.

The transplants, composed of soft, friable, pinkish yellow tissue, appeared similar to the primary tumor, except for a greater number of mitotic figures and absence of any bile ducts. Special stains revealed abundant glycogen and neutral fat in the cytoplasm of the tumor cells. Alkaline phosphatase was absent. Significant differences from normal liver cells have been noted in the Golgi apparatus and in the mitochondria of the tumor cells. In normal hepatic cells the mitochondria vary from short, plump rods and spheres at the periphery to fine, tenuous filaments at the center of the lobule. The mitochondria present in transplants of this tumor were practically all small spheres. The Golgi apparatus of normal liver cells always shows a peripheral distribution near the bile capillary, while that of the tumor cells, though large in amount, appeared to be more condensed and formed a mass situated nearer the nucleus.

A transplantable hepatoma arising spontaneously in a strain CBA mouse has been described (Strong, L. C., and Smith, G. M. *Am. J. Cancer,* 1936, 28, 112–114). To our knowledge, the current case is the first reported transplantable spontaneous hepatoma in a mouse of the C_3H strain. The tumor is being maintained at the National Cancer Institute and is designated as mouse hepatoma 98/15.

INTRATESTICULAR HEMORRHAGE: A BIRTH TRAUMA. Bela Halpert, New Orleans, La.

Abstract. Routine gross and microscopic examination of the testicles of newborn infants at necropsy led to the conclusion that quite extensive intratesticular hemorrhages occur rather frequently as an injury sustained during birth in cases where the head is the presenting part.

SPONTANEOUS CEREBELLAR HEMORRHAGE (REPORT OF FIFTEEN CASES).* Nathan Mitchell and Alfred Angrist (by invitation), Jamaica, N. Y.

Abstract. Fifteen cases of spontaneous cerebellar hemorrhage were encountered in a series of 3,881 autopsies in a 5-year period, during which interval 100 instances of hemorrhage into the cerebrum were noted. The erroneous impression exists that cerebellar hemorrhage is a rare lesion. Only 109 cases of spontaneous cerebellar hemorrhage were found in the literature, and the reported incidence, as compared to cerebral hemorrhage, varies from 0.9 to 10 per cent. It is of interest to note that the ratio of cerebellum to cerebrum by weight is 12+ per cent.

The clinical syndrome of cerebellar hemorrhage is a variable one and this, in part, accounts for the impression of a low incidence. Such patients either die suddenly, particularly when rupture into the ventricular system or subarachnoid space complicates the picture, or they recover. In the former event, it is difficult or impossible to discover at autopsy the exact origin of the hemorrhage. In 15 cases, 6 died suddenly or were found dead, in contrast to only 3 found to have died suddenly in the 109 reported cases. A characteristic neurological syndrome such as occurs in cerebral hemorrhage is rather uncommon. Associated lesions included cardiac hypertrophy, uremia, and leukemia; these are identical with the factors commonly associated with cerebral hemorrhage. Cerebellar hemorrhage represents a lesion comparable to cerebral hemorrhage in incidence and identity of contributory pathological factors, with a tendency to extremes in the clinical picture.

THE OCCURRENCE OF A DOUBLE ZONE PHENOMENON IN ANTI-HUMAN TISSUE SERUM. Anderson Nettleship, Bethesda, Md.

Abstract. Through the use of an optimal precipitin method it was possible to show a double zone phenomenon in duck anti-human kidney serum. This double zone

* This article will appear in the September issue of the *American Journal of Pathology.*

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THE AMERICAN JOURNAL OF PATHOLOGY

VOLUME XVIII SEPTEMBER, 1942 NUMBER 5

THE GOORMAGHTIGH CELLS IN THE NORMAL AND DISEASED HUMAN KIDNEY*

THEIR POSSIBLE RELATIONSHIP TO RENAL HYPERTENSION

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Recent experimental studies of hypertension in animals have focused attention anew on certain special structures present in the mammalian kidney.[1-6] These are the cell groups situated in the wall of the preglomerular arteriole, the juxtaglomerular apparatus of Goormaghtigh: and the specialized epithelial cells, the macula densa, present in the distal convoluted tubule. Indeed, the suggestion has been made that the juxtaglomerular apparatus may be concerned with the elaboration of a pressor substance.[7-14] During the past few years I have been studying these cell groups at the vascular pole of the glomerulus in the kidneys of human patients with and without hypertension, and wish to report certain findings which may have a bearing on this subject.

MATERIAL

Approximately 400 human kidneys were obtained either from the living patient or at autopsy. As soon as possible, usually within from ½ to 4 hours after removal, the tissues, cut into small blocks, were fixed in Zenker's or Bouin's solution. Sometimes Zenker's solution was injected into the renal artery while the organs were still *in situ,* to obtain more prompt fixation. Thin slices were dehydrated, cleared, embedded in paraffin and sections were cut serially at 4 to 6 μ. These were stained by the following methods: Goldner's modification of Masson's trichrome method, Mallory's aniline blue, Weigert's hematoxylin, Heidenhain's azan-carmine and Lillie's Biebrich-scarlet-red. Sometimes other stains were employed. Preparations stained by hematoxylin and eosin were also made and served as controls.

* Received for publication, December 17, 1941.

Intranuclear and cytoplasmic inclusion bodies were found in various organs in 12 per cent of 50 patients suffering from this pancreatic disease, an incidence no greater than the average for all autopsies performed in this laboratory. Evidence obtained from postmortem examinations, analysis of pancreatic enzymes and experimental studies supports the suggestion of Wolbach that the pathogenesis of this pancreatic lesion resides in the production of an abnormal pancreatic secretion. Inspissation of this material causes obstruction which leads to dilatation of ducts with connective tissue replacement of atrophied parenchyma, and to pancreatic achylia. The cause for this suggested disturbance to the secretory mechanism in the pancreatic acini remains to be demonstrated.

THE STOMACH IN PERNICIOUS ANEMIA. Alvin J. Cox, San Francisco, Calif.

Abstract. In six autopsied cases of pernicious anemia of different duration and treated for various lengths of time the stomachs showed relatively little postmortem alteration and could be studied thoroughly after being stretched flat and fixed. All showed extensive mucosal changes in the body and fundus like those described by Meulengracht and others in cases of pernicious anemia. The changes were sharply delimited from the pyloric zone and could be distinguished from those in other types of so-called "gastritis." The character of the mucosal change suggests a specific injury of the specific secretory cells of the body and fundus of the stomach with repair by less differentiated epithelial cell types. There is no evidence to suggest a return to normal in the cases which had received prolonged therapy. A case of long-standing sprue with severe macrocytic anemia showed no significant lesions. It is concluded that the gastric changes are not the result of anemia and are not necessarily associated with this type of anemia; but the high incidence of characteristically altered gastric mucosa in patients with pernicious anemia suggests a true relationship which, in the light of recent clinical experimentation, may be etiological. Other examples without gastric lesions may represent similar anemias resulting from different causal factors.

Since recent experimental work in dogs and rabbits by Goormaghtigh,[7-9] and in rabbits by Dunihue and Candon [11] and Dunihue,[12] seems to indicate that certain changes, such as hypertrophy and hyperplasia of these cells with concomitant alterations of the granular contents of the cytoplasm, may occur in experimental renal ischemia, an attempt was made to compare human kidneys which were definitely pathological with apparently "normal" kidneys. A large number of cases of so-called benign progressive arteriolar nephrosclerosis, three cases of malignant nephrosclerosis and three cases of pyelonephritis with secondary vascular changes, all with clinical hypertension, were examined.

It was found that there were definite hypertrophy and hyperplasia of Goormaghtigh cells in the kidneys of patients with chronic pyelonephritis with vascular changes, moderately advanced and advanced arteriolar nephrosclerosis and one case of malignant nephrosclerosis, all with clinical hypertension. However, in contrast to the experimental animal, there were no detectable associated alterations in their cytoplasm. Granules were present in scanty amount but they were neither more definite nor distinct than they were in the Goormaghtigh cells of normal kidneys.

In addition, in those cases where such severe vascular lesions were present that the cells which normally form the vessel wall underwent atrophic and degenerative changes, the Goormaghtigh cells remained remarkably well preserved. Moreover, in many of the interlobular arteries whose walls were markedly sclerosed and even hyalinized, groups of well preserved Goormaghtigh cells were frequently observed in or around the damaged media.

Comment

From these observations, together with those made by others, it now appears justifiable to add to the known structures of the normal human nephron two others which are regularly found in the renal cortex. These are the Goormaghtigh cells of the arteriolar walls and the macula densa of the distal convoluted tubule.

The nature of the Goormaghtigh cells is uncertain. Ruyter,[1] Oberling,[2] Goormaghtigh [3] and Zimmermann [4] noted that they were usually afibrillar and occurred constantly in the media of the arterioles. They considered them to be specially differentiated leiomyoblasts. Although in general they were afibrillar, Ruyter and Goormaghtigh found some cells which contained a few myofibrils, for which Goormaghtigh later coined the adjective "paucifibrillar." Ruyter, studying these cells in the mouse, was the first to demonstrate mitochondrial granules in their protoplasm, while Goormaghtigh [3] somewhat later found granules in

Observations

In the human kidney, as in the kidneys of animals, a group of distinct cells is seen at the vascular pole of many but not all glomeruli. These cells are located between the afferent and efferent arterioles, frequently appearing to surround, usually eccentrically, the afferent vessel. Occasionally they extend a short way into the glomerulus along a capillary of the tuft. In this location they maintain a close relationship to the macula densa of the distal convoluted tubule from which no visible membrane seems to separate them. These cell structures are not confined to the vascular pole of the glomerulus. They are found most frequently in this situation but they also occur singly or in smaller groups along the interlobular artery and even the terminal artery. In the afferent arteriole they often form the entire vessel wall, being separated from the lumen by endothelial cells only. In the larger vessels they occupy the outer third of the media, often being dispersed irregularly among the smooth muscle cells, or are present only in the adventitia.

In recognition of the man who first studied and described these cells most extensively, I am calling them, as noted elsewhere,[13,14] "Goormaghtigh cells." In the human kidney they are large, polygonal and have indistinct cell outlines. Although they are usually closely packed, delicate argyrophilic fibrils, possibly of nervous origin, can often be detected between them. Their cytoplasm is scanty, usually appears vacuolated and sometimes contains a few fine fibrillar structures, although it is commonly afibrillar. Occasionally a few, fine fuchsinophilic granules, very difficult to differentiate from broken myofibrils, are present. The nucleus is small, round and vesicular, and usually situated in the center of the cell but sometimes found eccentrically. Frequently a clear halolike zone surrounds it.

Because of the fact that Goormaghtigh cells are so frequently situated near or among smooth muscle cells and occasionally contain broken myofibril-like structures in their cytoplasm, it has been suggested that they originate from muscle cells. Indeed, I was able to find what appeared to be actual transitional stages from one cell type to the other. I am therefore inclined to believe that they originate from smooth muscle cells.

The Goormaghtigh cells seem to be intrinsic parts of the human kidney, although their number may vary in individual cases. The youngest patient in whom I have observed them clearly was a boy of 3 months. They have also been found in the kidneys of a woman 78 years of age. No difference as to sex and race was found. They are more readily seen in the outer portions of the cortex, while in the deeper layers their appearance is less regular.

physiology and function (Okkels and Péterfi [28] and Okkels [29]). However, my observations in pathological kidneys as well as experimental work by others [7-9, 11, 12] point to the possibility of a third hypothesis which is incompatible with both of the foregoing.

This hypothesis was first propounded by Goormaghtigh [7] as a result of certain studies made in 1939. In the course of experiments on renal hypertension in cats and rabbits, he drew attention to what he called the endocrine character of the afibrillar cells. This was a totally new concept. In four rabbits, in particular, he observed a variable number of secretory granules in the cells of the juxtaglomerular apparatus. At first these cells were afibrillar; then they became loaded with acidophilic granules which finally turned toward basophilia. From these observations he concluded that:

"L'ischémie rénale provoque l'hypertrophie et la multiplication de certaines cellules de la média des artérioles du rein qui normalement sont dépourvues de myofibrilles et possèdent chez le lapin des caractères cytologiques de cellules endocrines. A condition d'être assez accentuée, elle provoque la transformation des cellules musculaires à myofibrilles en cellules afibrillaires qui deviennent toujours granuleuses chez le lapin et parfois aussi d'une manière plus discrète chez le chien. Quand l'ischémie dure plusieurs mois, de nombreux éléments afibrillaires des artérioles rénales du chien deviennent granuleux et acquièrent des caractères cytologiques endocrines comme chez le lapin." [Experimental renal ischemia produces hypertrophy and hyperplasia of certain cells of the media of the renal arterioles which normally do not have myofibrils and which in the rabbit possess cytological characteristics of endocrine cells. If the ischemia is well marked, it produces the transformation of the myofibrillar smooth muscle cells into afibrillar cells which always become granular in the rabbit and sometimes to a slighter degree in the dog. When the ischemia lasts for several months, numerous afibrillar elements of the renal arterioles of the dog become granular and take up endocrine cytological characteristics, as seen in the rabbit.]

It should be noted, however, according to Goormaghtigh,[7] that in the rabbit these granular changes are found chiefly in the afibrillar cells of the superficial glomeruli, while deeper in the cortex the granularity is much less pronounced.

This hypothesis, based as it is on morphological changes alone, becomes important only in the light of physiological experiments on renal hypertension. The earliest investigations in the latter field were those of Tigerstedt and Bergman,[30] who, in 1898, isolated from the cortex of various mammalian kidneys a hypertensive substance which they called "renin." Nearly 40 years later, Prinzmetal and Friedman,[31] working on dogs and cats, and Harrison, Blalock and Mason,[32] working on dogs, confirmed the work of Tigerstedt and Bergman. Many others [33-46] have done the same and isolated other additional substances, of which the importance in hypertension is today generally recognized. Although there is as yet no agreement as to the name or even the identity of the

the afibrillar cells of the renal arterioles of the cat. Some investigators, notably Ruyter [1] and Schumacher,[15] thought that the afibrillar cells were epithelioid in character, but today it is generally considered that they are actually transformed smooth muscle cells. I am inclined to agree with this interpretation, although often it was extremely difficult to differentiate between broken-up myofibrils and separate acidophilic granules in paucifibrillar cells. Indeed, there seems to be good evidence for the transition of the smooth muscle cells into paucifibrillar and afibrillar cells. Their location in the arteriolar wall as well as their cytological characteristics favor such a transition.

Although Goormaghtigh cells were actually found some distance away from the glomerulus—in the interlobular artery and the terminal artery, for example—it would appear that their usual location at the vascular pole and their close proximity to the macula densa must be of significance. Thus it could readily be assumed that these cell groups and the macula densa might form a unit which had a special physiological function to perform.

In his early work Goormaghtigh [3] thought that the physiological significance of the afibrillar cells was identical with that of the epithelioid cells described in arteriovenous anastomoses occurring in other parts of the body (Schumacher,[15] Vastarini-Cresi,[16] Grosser,[17] Masson,[18-20] Clara,[21] Grant [22] and Popoff [23]). He reasoned that the afibrillar leiomyoblasts of the renal arterioles, situated as they are between a contractile arteriolar segment and a capillary network, could bring about the temporary exclusion of the glomerular loops from the renal circulation. This, of course, would make possible diversion of the blood through aglomerular arteriovenous shunts (Oliver and Luey,[24] Dehoff [25] and Spanner [26]). Moreover, the observation of an intricate sympathetic nerve plexus among these cells led Goormaghtigh to believe that in them could originate a reflex which controlled the contraction or relaxation of the smooth muscle cells of the media. The smooth muscle cells would thus control the blood supply of the glomerulus.

Clara,[27] on the other hand, thought that turgescence of these "epithelioid" cells could bring about closure of the afferent arteriole while their relaxation would open the vessel again into the glomerulus. He believed that the physiochemical condition of the glomerular filtrate in the distal convoluted tubule provided the stimulus for the closing and opening mechanism via the macula densa, which acted as a "receiving" unit.

The intimate relationship of Goormaghtigh cells and macula densa at the vascular pole of the glomerulus in normal human kidneys could support either one of these hypotheses. Moreover, either hypothesis could be reconciled with much of the recent experimental work in renal

hyperplasia of Goormaghtigh cells is so pronounced, may be of any significance? Further work is certainly necessary to correlate these experimental findings with the observations made in the kidneys of hypertensive patients. In these organs the Goormaghtigh cells lack the morphological characteristics of endocrine function as defined by Goormaghtigh.

Thus in the absence of definitely demonstrable cytoplasmic granules in the Goormaghtigh cells of kidneys from patients with hypertension, the hypertrophy and hyperplasia of these cells appear at present to be the only evidence suggestive of an endocrine function which could be related to the production of a renal pressor substance.

Summary

Four hundred kidneys, removed either at operation or at autopsy, were examined and Goormaghtigh cells were regularly found at the vascular pole of those glomeruli which were situated in the outer part of the cortex. They appeared to be intrinsic parts of the human kidneys. They were situated close to the macula densa of the distal convoluted tubules. They also occurred in and along the wall of the afferent arteriole, the efferent arteriole, the interlobular artery and the terminal artery. Their location and their cytological characteristics would indicate that they are probably transformed smooth muscle cells of the vascular media. Although their close relationship to the macula densa suggests that they provide an opening and closing mechanism for the access of the blood to the glomerular tuft, my findings as well as recent work on experimental hypertension of renal origin cast considerable doubt on this theory and strongly suggest the possible endocrine character of the Goormaghtigh cells.

Note: I wish to express my gratitude to Dr. Arthur W. Wright, Professor of Pathology and Bacteriology, Albany Medical College, for his valuable assistance in carrying out this work.

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renal hypertensive substance or substances, there is sufficient experimental evidence for their existence in the renal cortex and in the cortex alone. Since renin is found only in the renal cortex and since it is in this part of the kidney that the afibrillar cells become granular and show "endocrinic characteristics," Goormaghtigh suggested that these cells may be concerned with the production of the pressor substance.

As far as animal experiments are concerned, Dunihue and Candon,[11] and Dunihue,[12] who have produced hypertension by using Drury's [47] method and Page's [48] cellophane method, have recently been able to confirm Goormaghtigh's latest observations in the rabbit.

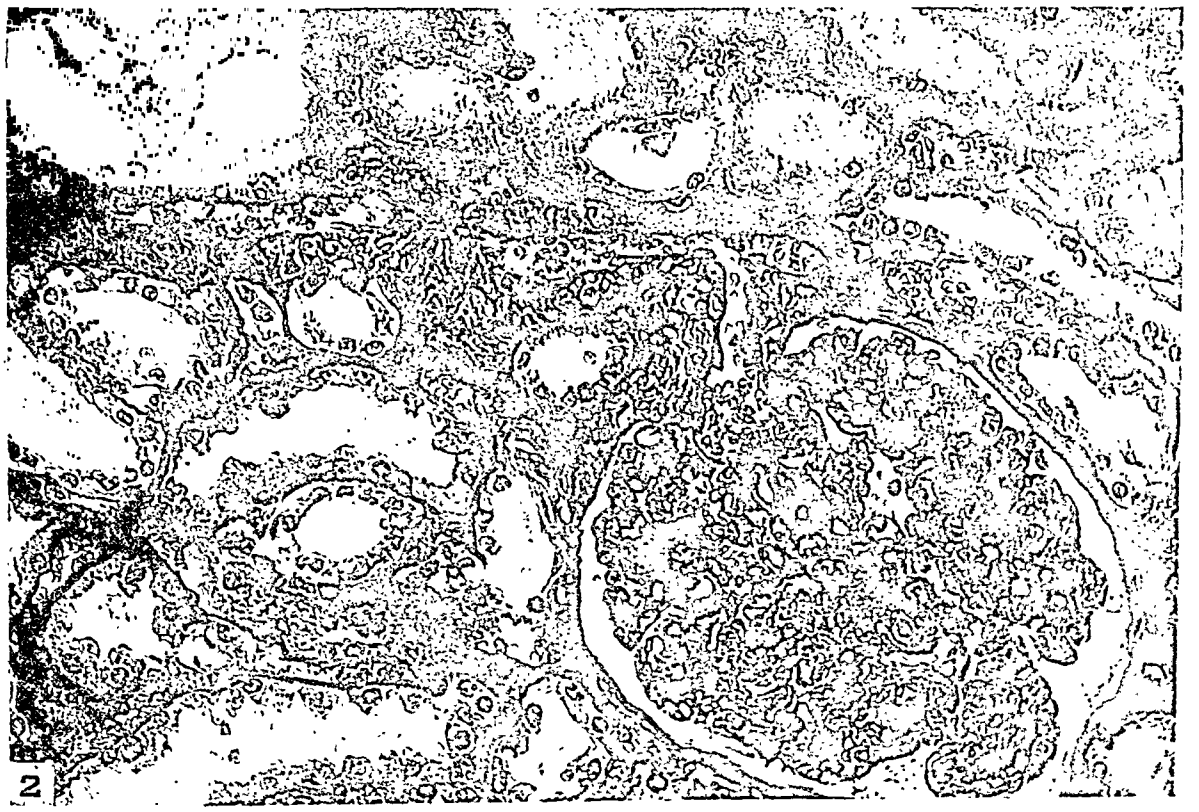
I am indebted to Dr. Dunihue for permitting me to examine some of his sections. They show beautifully, in the afibrillar cells, the granular changes which are found in association with experimental hypertension. However, in the human kidney the demonstration of intracellular granules is much more difficult than in the experimental animal. It is absolutely essential for the demonstration of these intracellular granules to obtain very fresh material, which, in the human, is not always possible. From my own observations in human kidneys as well as from those of others in the kidney of the dog, it seems that species differences exist in the Goormaghtigh cells which have now been described in most vertebrates.[49] It has already been mentioned that in the cases of hypertension with renal arteriolar damage which I studied there was uniform hypertrophy and hyperplasia of the Goormaghtigh cells, particularly in the afferent arterioles, but that the granular changes described in the experimental animal were not found. In addition, analogous to the findings of Goormaghtigh and Handovsky,[50] who fed dogs large doses of vitamin D_2 (calciferol) and noted that the afibrillar cells remained undamaged long after severe sclerosis and necrosis of the renal arteriolar wall had set in, in the kidneys of hypertensive patients well preserved Goormaghtigh cells were found in severely damaged arterioles.

These findings are significant in the light of the experiments which demonstrated the presence of a pressor substance in the cortex of the normal kidney. In experimental renal hypertension this pressor substance is increased and appears in the venous blood of the kidney. This suggests strongly that the hypertrophied and hyperplastic Goormaghtigh cells of the afferent arterioles of the glomerulus might be its source. Moreover, it is generally assumed that the vasoconstrictor effect of the pressor substance acts directly on the smooth muscle cells of the arteriolar media. Since it is known that this constriction is particularly pronounced in the efferent arterioles, does this suggest that the proximity of this vessel to the afferent arteriole, in which hypertrophy and

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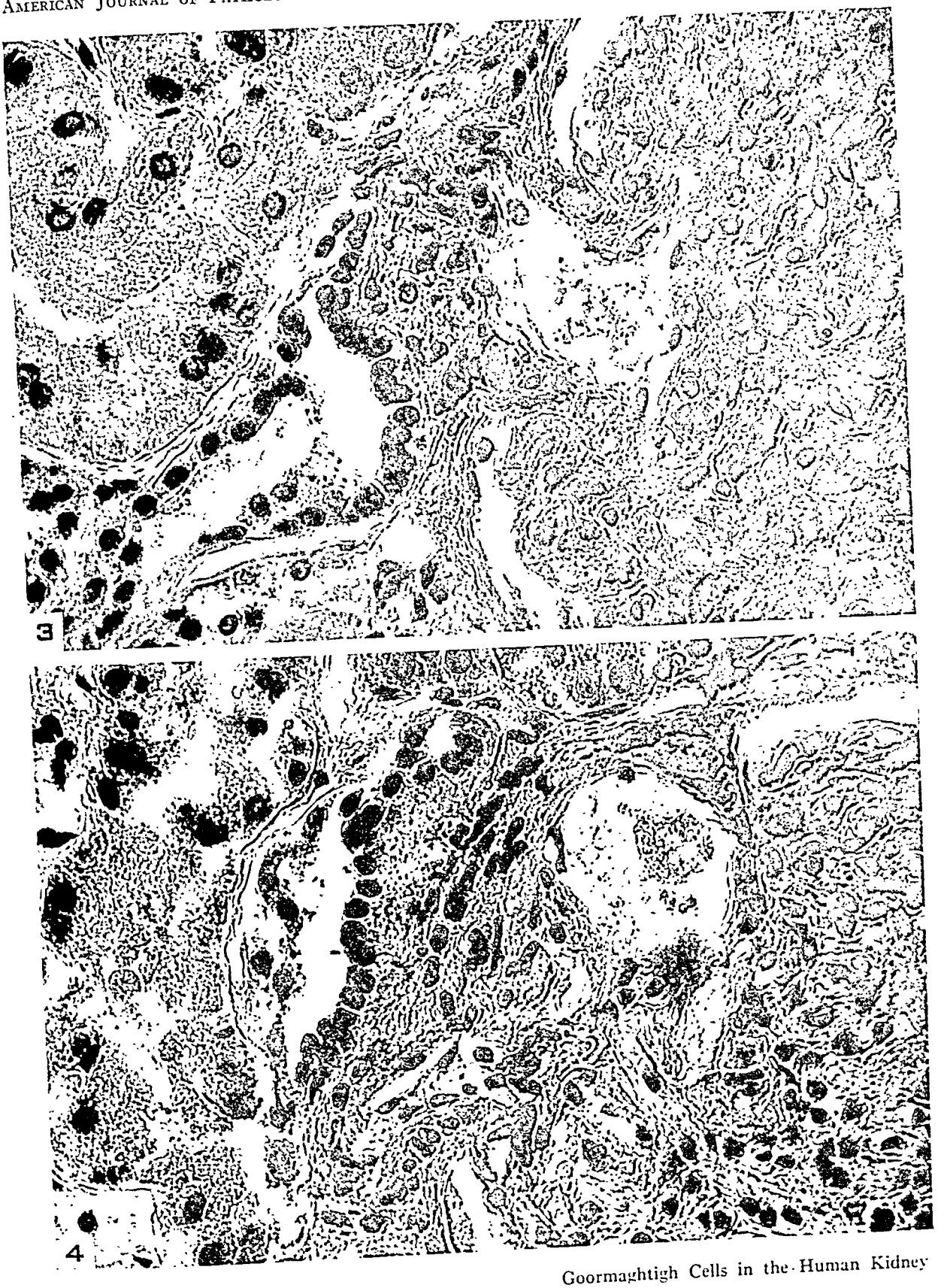
Kaufmann Goormaghtigh Cells in the Human Kidney

DESCRIPTION OF PLATES

PLATE 114

FIG. 1. Vascular pole of a glomerulus with juxtaglomerular apparatus and adjacent macula densa of a distal convoluted tubule. The Goormaghtigh cells of the juxtaglomerular apparatus and the tall epithelial cells of the macula densa are in close contact. The kidney was from a negro, 25 years old, who died of tuberculous meningitis. There was no renal pathology. Hematoxylin and eosin stain. × 545.

FIG. 2. Glomerulus, afferent arteriole and interlobular artery from a kidney of a white man, 40 years old, with moderate hypertension for several years. There is eccentric thickening of the arteriolar wall, particularly at the vascular pole of the glomerulus, and a moderate degree of hyalinization of the capillaries of the glomerular tuft. Mallory's aniline blue and Weigert's iron hematoxylin stain. × 275.



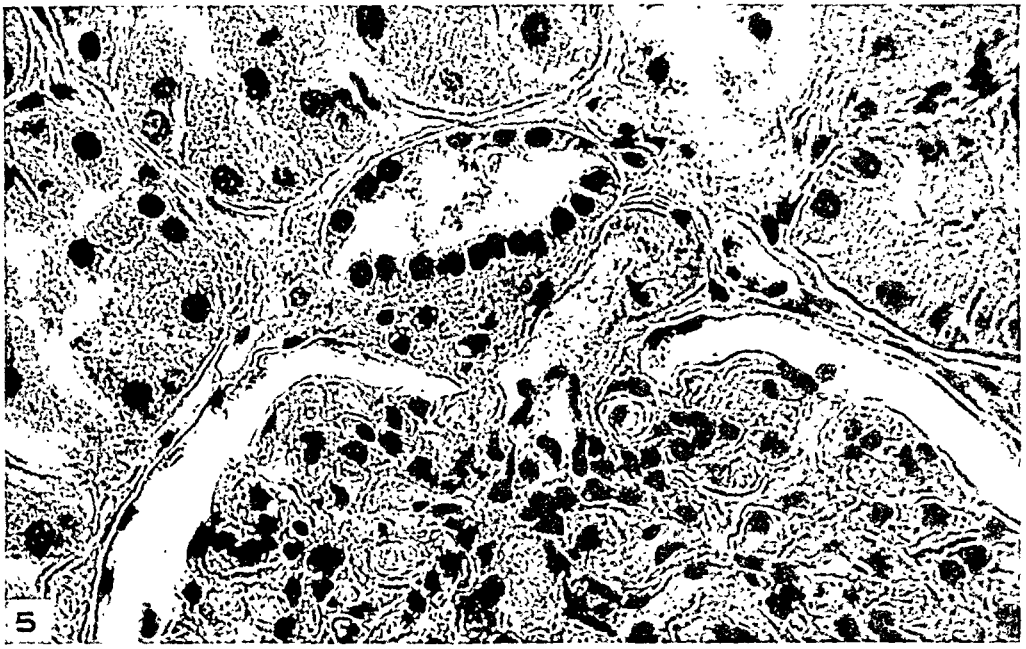
Goormaghtigh Cells in the Human Kidney

Kaufmann

PLATE 115

FIG. 3. Beginning sclerosis in the wall of an afferent arteriole with moderate hypertrophy and hyperplasia of Goormaghtigh cells. This was from the kidney of a white woman, 78 years old, with long-standing moderate hypertension. A well outlined macula densa of a distal convoluted tubule may be seen close to the afferent arteriole. Goldner's modification of Masson's trichrome stain. × 570.

FIG. 4. Another glomerulus, Goormaghtigh cells and macula densa, from the same patient as Figure 3. There is progressing sclerosis of the afferent arteriole and beginning disintegration of Goormaghtigh cells. Goldner's modification of Masson's trichrome stain. × 570.





Kaufmann Goormaghtigh Cells in the Human Kidney

PLATE 116

FIG. 5. A third glomerulus. Goormaghtigh cells and macula densa from the same patient as Figures 3 and 4. There is marked hyalinization of the wall of the afferent arteriole and almost complete disappearance of Goormaghtigh cells. Goldner's modification of Masson's trichrome stain. × 505.

FIG. 6. Interlobular artery from a kidney of a white man, 54 years old, with severe hypertension of several years' duration. (Blood pressure was 254/158 mm. of Hg on admission.) There is extreme sclerosis of the arterial wall with hypertrophied, hyperplastic and well preserved Goormaghtigh cells. Lillie's Biebrich-scarlet-red stain. × 505.

INTRANUCLEAR INCLUSIONS IN INFANCY*

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The question of the etiology and significance of inclusion bodies in the organs of infants has given rise to considerable speculation since Jesionek and Kiolemenoglou [1] first described them in 1904, in the lungs, kidneys and liver of an 8-month luetic fetus. In the same year, Ribbert [2] described similar structures in the parotid glands of two nonluetic infants and reported that he had previously (1881) observed them in a kidney of a luetic stillborn infant. In both reports it was concluded that these intranuclear inclusions most likely represented some one of the protozoa. Löwenstein,[3] in 1907, found intranuclear inclusions in both parotid glands in 4 of 30 infants and, in addition, described cytoplasmic inclusions. Pisanò,[4] in 1910, and Mouchet,[5] in 1911, also reported finding intranuclear inclusions in luetic infants. In the United States in 1910 and 1914, Smith and Weidman [6, 7] of Philadelphia described similar inclusions. These authors concluded that they were due to a protozoan to which they gave the name *Endamoeba mortinatalium*. No other important observations were made until 1920, when Jackson [8] described intranuclear inclusions in the salivary glands of guinea pigs, but these, too, were thought to be protozoa.

The protozoan theory was not discarded until 1921 when Goodpasture and Talbot [9] showed these inclusions to be products of modified cells, and compared them with the nuclear changes in the cutaneous lesions which Tyzzer [10] had described in varicella in 1906. It was in the same year (1921) that Lipschütz [11] reported the finding of intranuclear inclusions in the lesions of herpes in rabbits and man, and concluded that they represented specific reactions to a specific virus. However, in the following year, 1922, Müller [12] reported 3 cases of inclusions in the kidneys of infants and postulated that the "protozoan-like cells" represented degenerated tissue cells. In 1925, Von Glahn and Pappenheimer,[13] for the first time, reported the finding of similar inclusions in an adult. They described these lesions in the intestines, liver and lungs of a male, 36 years old, who died from ulcerative colitis, liver abscess and pneumonia. Walz,[14] in 1926, reported the case of a newborn infant with inclusions in the lungs, thyroid, liver, pancreas and kidneys, while Wagner,[15] in 1930, found inclusions in the sublingual gland, lungs, thyroid, liver, pancreas, kidneys and epididymis of a 2-week premature infant and inclusions in the parotid glands of 4

* Received for publication, December 26, 1941.

Postmortem Examination

Autopsy was performed, but no gross abnormalities were found in any of the organs.

Sections were taken for study from the cerebrum, medulla, pons and cervical cord. These were fixed in Zenker's fluid and stained with phloxine and methylene blue. The inflammatory process present was widespread in all of the sections studied with the exception of those taken from the cord. Throughout the cortex and subcortical white matter of the cerebrum were focal areas infiltrated by polymorphonuclear leukocytic cells together with moderate numbers of mononuclear cells (Fig. 1). In the white matter these areas were distinctly perivascular while those in the gray matter were more diffuse. In a few of these foci in the gray matter the tissue was partially necrotic, but in other similar foci in both the gray and white matter there was no obvious necrosis. In some of these areas intranuclear inclusions could be seen in the nerve cells. The walls of many of the blood vessels of the parenchyma were necrotic and were infiltrated by polymorphonuclear leukocytes and macrophages (Fig. 2). There was considerable perivascular cuffing by lymphocytes. One intranuclear inclusion was found in a cell in a vessel wall.

Focal lesions similar to those described were found throughout the medulla. However, these lesions appeared less acute as there were larger numbers of mononuclear cells and fewer polymorphonuclear cells. These lesions were especially prominent in the vagal nuclei and intranuclear inclusions were quite numerous there.

The intranuclear inclusions were most prominent in nerve cells adjacent to the lesions described (Figs. 3 and 4). In cells in which the inclusions were well developed, the nuclear chromatin was marginated, leaving a clear zone between the nuclear membrane and the inclusion. The inclusions were definitely acidophilic and were quite granular in appearance. These inclusions did not resemble in any way those which are described as occurring in the nuclei of ganglion cells in poliomyelitis and other virus diseases.

At the time of autopsy, the nature of these lesions was not suspected and so no effort was made to recover a virus. However, the morphology of the lesions and particularly of the inclusion bodies strongly suggests a herpeslike virus as the etiological agent in this case. The negative bacterial cultures of the spinal fluid support this viewpoint. The vascular lesions deserve emphasis, as these may account for the large areas of necrosis. This is the fourth case to be reported, as far as can be determined, in which intranuclear inclusions similar to those of herpes have been found in the brain. Two cases were reported by

infants. In 1932, Farber and Wolbach [16] reported by far the largest single series—22 cases in which the inclusions were found in the submaxillary glands alone, together with 2 cases in which inclusions were present in the viscera. In this series the submaxillary glands were studied in a series of 183 postmortem examinations on infants and the incidence of the inclusions was 12 per cent. These investigators described cytoplasmic inclusions which were frequently found in conjunction with the intranuclear inclusions.

Subsequently Rich [17] and also McCordock [18] and McCordock and Smith [19] have reported the finding of intranuclear inclusions associated with pertussis, and Goodpasture, Auerbach, Swanson and Cotter [20] have recently described 5 cases of virus pneumonia in infants in which intranuclear inclusions were present. These findings will be discussed in detail in a later paragraph.

In 1940, Vidari [21] reported a case with "protozoanlike cells" in the lungs, thyroid, liver and kidneys, associated with icterus neonatorum and erythroblastosis, and stated there were 5 similar cases in the literature.

Part 1

The patient, a male child of 3 years, was taken to the hospital because of convulsions and colonic spasms.

Little was known concerning the past history except that there had been no recent acute illnesses. Several siblings were living and well. On July 1, 1933, the patient fell from a coaster wagon, receiving a severe blow on the lumbar region, but except for a bruise there was no evidence of injury. Two days later the child fell from a porch and cried for a considerable period of time but moved about normally. On July 9, 1933, he suffered from what was termed a "convulsion," trembling and crying for short intervals. Following this convulsion, the patient was examined by a physician who was unable to find any evidence of disease. Three days later more convulsions took place and the child was hospitalized.

Physical examination at the time of admission showed a well nourished boy, lying on his back in bed, in a semistuporous condition. The temperature was normal. He could be roused by speech and by shaking but was apparently disoriented. Examination of the lungs, heart and abdomen presented no abnormalities. The pupils reacted to light. The tongue protruded in the midline without apparent tremor. The lips twitched constantly but were not drawn to either side. The right abdominal and the cremasteric reflexes were absent. The knee jerks could be elicited but were sluggish. The Kernig and Babinski reflexes were negative.

The convulsions became steadily more severe during the patient's 2 days in the hospital. He was incontinent, comatose and unable to retain food or fluid. On the second hospital day, marked swelling appeared over the left frontal region which, in a very short time, involved the lids of the left eye to such a degree that they could not be opened. The temperature remained normal until shortly before death when it rose sharply to 105° F. In addition, the patient became rigid for a short time preceding death.

A lumbar puncture was performed. The spinal fluid was not under increased pressure. Eight cc. of blood-tinged fluid was removed. Cultures of this fluid were negative.

one or several dense, granular, round, basophilic masses suggesting chromatin could usually be found. In many cases there was beading of the nuclear membrane by dense, irregular, granular, basophilic material which was interpreted as being remnants of nuclear material (Fig. 5).

In many of the cells containing intranuclear inclusions, cytoplasmic inclusions were found as well. These were especially prominent in the kidney and pancreas. They appeared as irregular, finely granular, basophilic masses lying between the cell and nuclear membranes.

Occasionally, a few lymphocytes were found lying near the cells containing inclusions; otherwise, there was no apparent reaction in the surrounding tissue.

Microscopically, the areas of softening in the brain were found to be areas of marked necrosis with mononuclear cell infiltration. One intranuclear inclusion similar to those found in the other organs was found in the brain but it was not in or near an area of necrosis (Fig. 6). This finding is of considerable interest, as it is the first time such inclusions have been described as occurring in the brain.

This case is presented as an example of the so-called "inclusion disease" of infants; cases in which the inclusions are so widespread in the body are distinctly rare. Only 25 cases have been reported in which inclusions have been found in organs other than the parotid gland and in only 11 have they been found in several of the viscera, as in the present case. In the remaining 14 cases the inclusions were found only in single organs and in this group they were present most frequently in the lungs, kidneys, or liver.

These inclusions are morphologically indistinguishable from those found in the salivary glands of children and from those in the submaxillary glands of guinea pigs.[25, 26] However, while the disease of the submaxillary gland in guinea pigs has been shown to be due to a transmissible virus, no such results have been obtained in children.

Part III

Intranuclear inclusions were found in the lungs in four, and in the salivary glands in two autopsies of infants suffering from clinical whooping cough. All cases presented morphological evidence of pertussis.[27] Four of these infants died in from 2½ to 5 weeks following the onset of symptoms. No history could be obtained regarding the other two patients. It was felt that the period of time which elapsed from the onset to the time of death could be of little significance as an indicator of the presence of inclusions, as 31 cases were examined which fell into the same range of time without finding evidence of

Dawson,[22, 23] one in 1933 and the second in 1934; and the third case was reported by Smith, Lennette and Reames [24] in 1941. This latter group of investigators, in addition to demonstrating the intranuclear inclusions, were able to recover and identify the virus of herpes simplex.

Part II

The patient, a female infant, 14 days old, was born at the Boston City Hospital on October 24, 1939. The mother, a 19-year-old primipara, was followed throughout her pregnancy in the out-patient department and appeared to be in excellent health. The pregnancy was uneventful. The Hinton test was negative. She had suffered from measles and chickenpox as a child; otherwise the past history was not remarkable.

Labor was at term and was uneventful. The infant weighed 2355 gm. (4 lbs. 14½ oz.) at birth. When examined shortly following birth, the infant appeared to be normal in all respects except for its small size. Because of this the patient was placed in an incubator and also given oxygen for the first day. She was fed 10 oz. of breast milk per day. The patient did well until the 11th day when she began to pass many stools. On the following day, she began to vomit and to refuse food. Jaundice was noticed and occult blood was found in the stools. She lost ground rapidly, becoming dehydrated and moribund despite supportive treatment, and died in her 14th day of life.

Postmortem Examination

Postmortem examination was performed 4½ hours following death. The body was that of a poorly nourished female infant weighing 1780 gm. There was a marked icteric tint in the skin and sclerae. With the exception of the brain, the organs showed no gross abnormalities. The leptomeninges were not remarkable and the convolutions were not flattened. There were irregular areas of softening in the right putamen and in the left basal ganglia, whose diameter varied from 1 to 1.5 cm. These areas were bright yellow. Scattered throughout these areas of softening were smaller pearly white, plaquelike masses which appeared to be slightly firmer than the uninvolved parenchyma and which measured from 1 to 3 mm. in diameter. Lesions similar to the above were found throughout the occipital lobe to such a degree as to involve approximately one-third of the lobe. There were no gross abnormalities of the vascular system of the brain.

On microscopical examination there was no evidence of disease except the intranuclear inclusions in the lungs, liver, pancreas and kidneys and the lesions in the brain. These intranuclear inclusions, except for those in the brain, were always found in epithelial-lined spaces, a finding which was first emphasized by Farber and Wolbach.[16] They usually appeared as oval, dense, homogeneous acidophilic bodies located in the center of the nucleus. They were usually separated from the nuclear membrane by a halo or clear zone, although occasionally the zone would be obliterated by an especially large inclusion. In the clear zone,

Other arguments in favor of the virus theory are that whooping cough may be complicated by an encephalitis, as in the case of measles and influenza, and that patients recovering from an attack of whooping cough acquire a lasting immunity from the disease. Also, the highly contagious nature of pertussis is stressed. Rich, Long, Brown, Bliss and Holt [33] were able to produce in apes an upper respiratory infection, closely resembling the catarrhal stage of whooping cough, by the inoculation of bacteriologically sterile blood and filtered respiratory secretions obtained from early cases of human pertussis. In addition, they were able to transfer the agent from one ape to another by means of a bacteriologically sterile filtrate of the nasopharyngeal secretions. However, in other groups of apes they produced a condition simulating human pertussis in all respects by oral inoculation of pure cultures of Bordet-Gengou bacilli. Also, Macdonald and Macdonald [34] produced whooping cough by the instillation of pure culture of Bordet-Gengou bacilli into the noses and throats of four boys. A previous attempt to produce whooping cough in these children by the instillation of a culture filtrate was unsuccessful. Gallavan and Goodpasture [35] were able to reproduce the exact pathological lesions of human pertussis in the respiratory tract and lungs of the chick embryo by the infection of the chorioallantoic membrane and by the inoculation of the amniotic fluid with the Bordet-Gengou bacillus. They believed this to be conclusive proof that the pathogenic agent in whooping cough is the Bordet-Gengou bacillus. They did not consider the inclusion bodies to be of significance in pertussis because they are absent in the majority of cases and because they are found in infants not suffering from this disease. The suggestion has been made by Rich that the inclusions are the result of an initial virus infection which paves the way for the Bordet-Gengou bacillus. Rich also suggested the possibility of these inclusions resulting from aspirated herpes virus. It is evident that the etiology of these inclusions as well as their exact rôle in the pathogenesis of whooping cough is unknown.

Summary

1. Intranuclear inclusions similar to those of herpes are described in the brain of a child of 3 years.

2. A case of so-called "inclusion disease" of infants is reported.

3. Six cases are discussed in which intranuclear inclusions were found in the lungs or salivary glands of infants dying of pertussis.

Note: I wish to thank Dr. Leroy U. Gardner of the Saranac Laboratory for his kindness in supplying me the material for Part I.

inclusions. No other factor could be found which would give any indication of the presence of inclusions.

The inclusions found in the lungs were found in the cells lining the alveoli and bronchi and occasionally in the mucous glands of the bronchi and trachea. The cells containing the inclusions were approximately twice the size of the uninvolved cells and their cytoplasm was more basophilic. The inclusions themselves were acidophilic and were separated from the nuclear membrane by a clear zone. The nuclear membrane was often beaded by small fragments of chromatin. Large numbers of these cells lay free in the alveolar spaces. It was felt that these had become detached from the alveolar walls. The nuclei of a few of these cells contained two, or even three, inclusions. No cytoplasmic inclusions were found in the lung (Fig. 7).

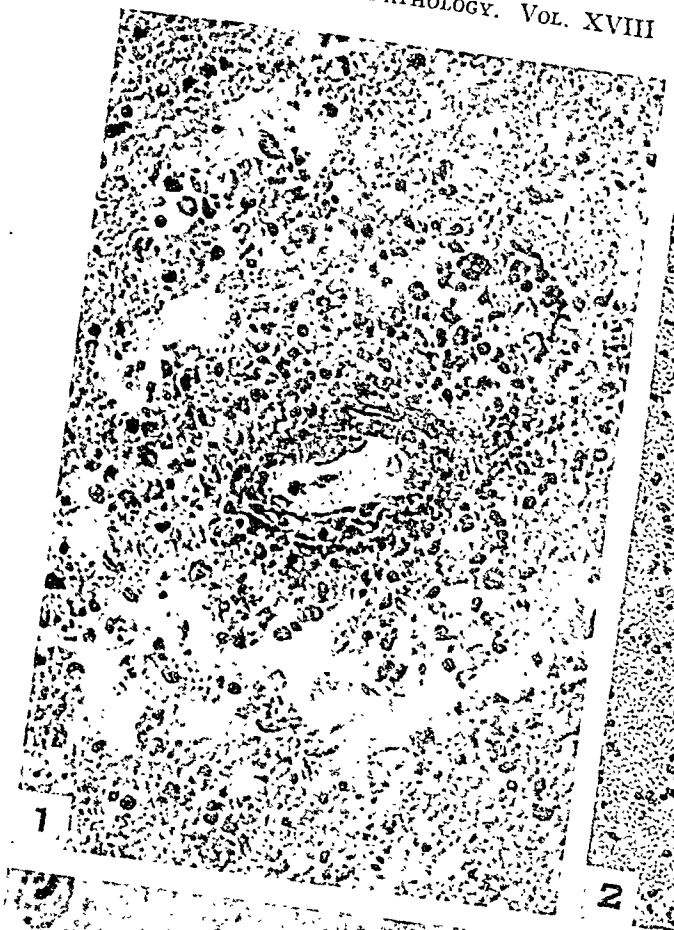
The intranuclear inclusions found in the salivary glands (Fig. 8) were similar to those found in the lungs. In addition, cytoplasmic inclusions were frequently present in these cells. These were basophilic, granular, spherical to elliptical bodies lying between the nuclear and cell membranes. Salivary glands were not examined in any of the 4 cases in which inclusions were found in the lung or in the larger group of cases of pertussis, described below, in which no inclusions were found. As a matter of interest, salivary glands from 25 infants were studied but no inclusions were present. The lungs from 31 cases of pertussis were studied without finding inclusions of any type. This represents an incidence of 16 per cent of intranuclear inclusions in the lungs or parotid material studied, and an incidence of 11 per cent in the lungs alone.

Feyrter,[28] in 1927, reported 1 case of intranuclear inclusions in the lungs of an infant dying of pertussis, while Rich,[17] in 1932, reported 5 cases. McCordock and Smith,[19] in 1934, reported the finding of these inclusions in the lungs in 18 of 40 autopsies; that is, in 45 per cent of children dying from pertussis. The finding of intranuclear inclusions in association with pertussis has prompted the suggestion that the etiological agent of pertussis is a virus. There are other reasons aside from the finding of inclusions to suspect that a virus may play some part in the pathogenesis of whooping cough. A reason frequently advanced is that the interstitial bronchopneumonia, which may accompany whooping cough, is similar to that seen complicating measles and epidemic influenza and to the pneumonia produced experimentally in animals by intratracheal injection of various types of virus. Less importance must be attached to this since Sprunt, Martin and Williams [29, 30] and Cullota, Marting and Liebow [31, 32] were able to produce interstitial pneumonia by injection of pure cultures of Bordet-Gengou bacilli in rabbits, mice and ferrets.

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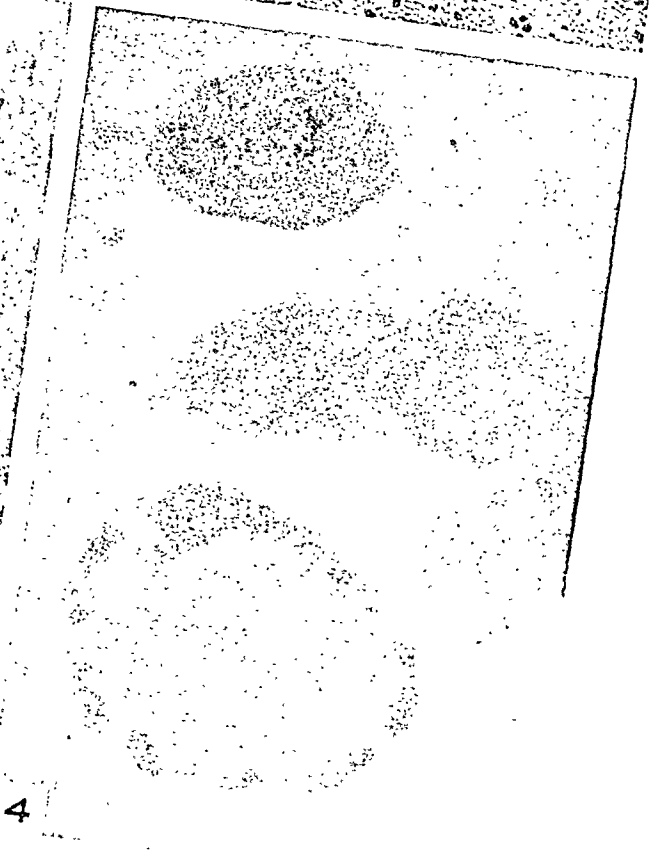
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Kinney

Intranuclear Inclusions in Infancy

DESCRIPTION OF PLATES

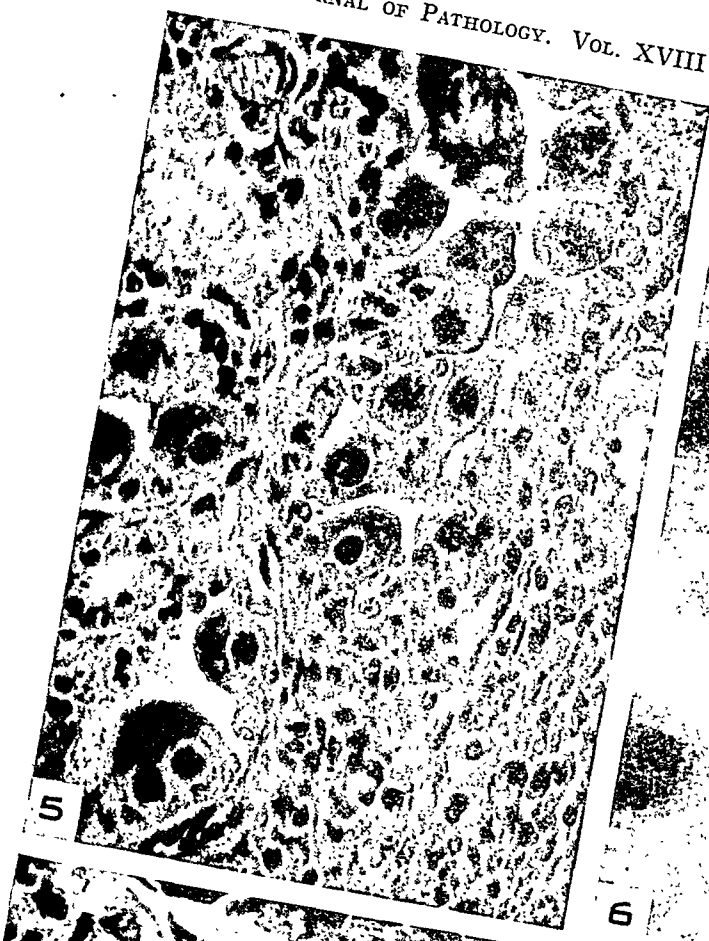
PLATE 117

FIG. 1. Cerebrum. Area of necrosis infiltrated by polymorphonuclear cells and monocytes. × 450.

FIG. 2. Cerebrum. Blood vessel with marked lymphocytic cuffing. × 340.

FIG. 3. Cerebrum. Intranuclear inclusions in nerve cells. × 1700.

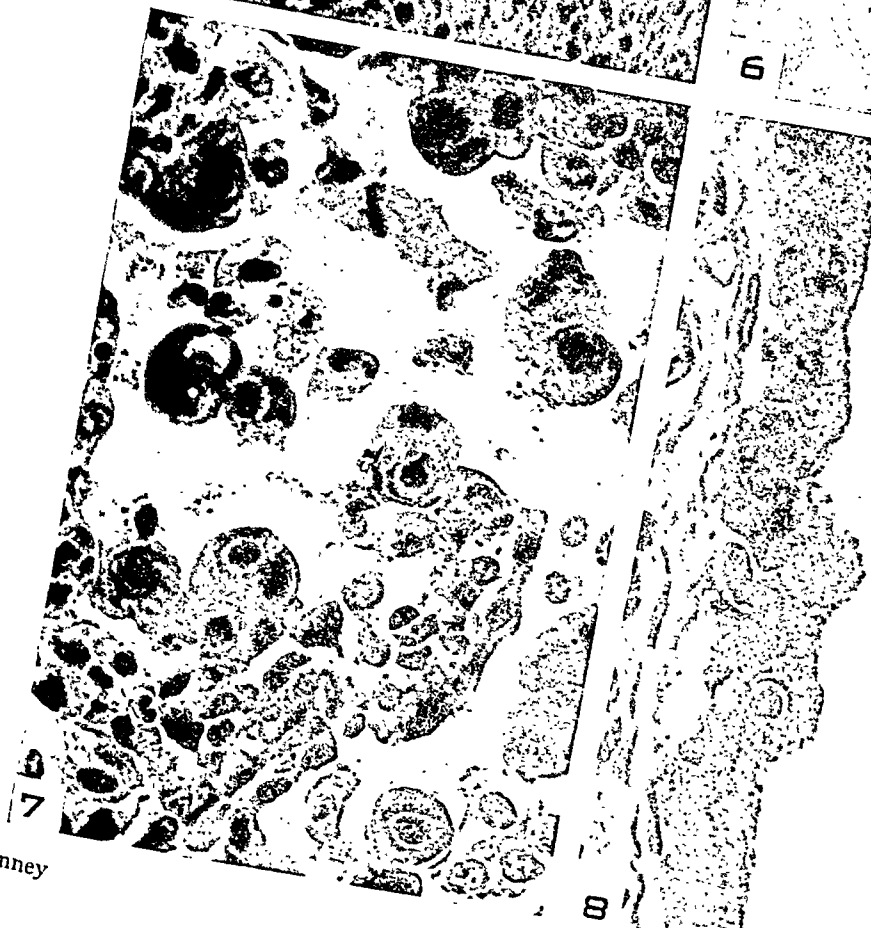
FIG. 4. Cerebrum. Single intranuclear inclusion. × 4230.



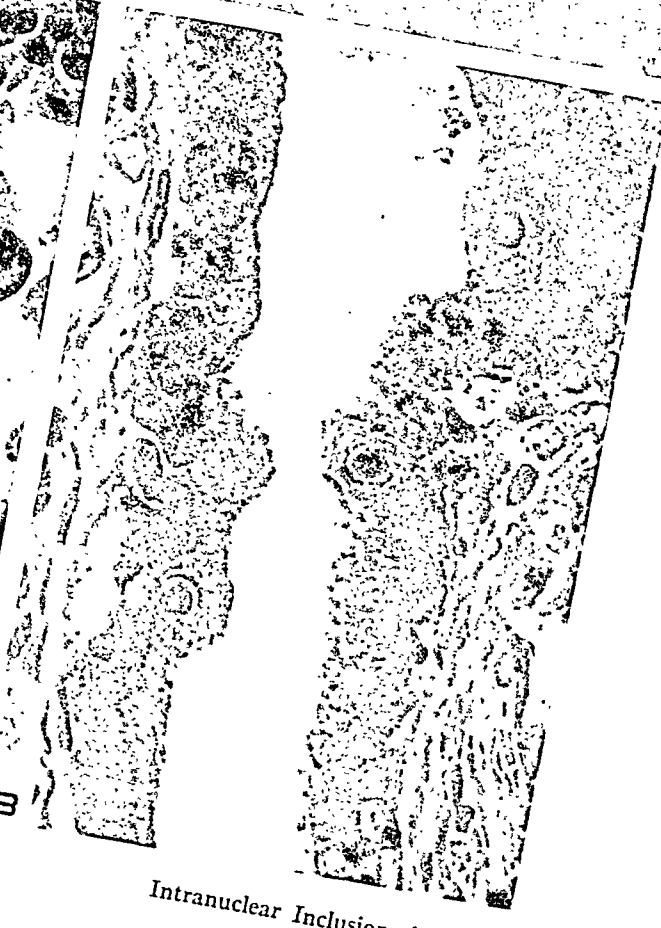
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Kinney

Intranuclear Inclusions in Infancy

PLATE 118

FIG. 5. Kidney. Low-power photomicrograph illustrating both intranuclear and cytoplasmic inclusions. × 500.

FIG. 6. Cerebrum. Single intranuclear inclusion. × 1300.

FIG. 7. Lung. Many intranuclear inclusions. × 500.

FIG. 8. Salivary gland. Intranuclear inclusions in duct epithelium. × 500.

PATHOLOGICAL CHANGES IN LISTERELLA INFECTION, PARTICULARLY OF THE EYE *

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During the course of an investigation on the genus Listerella [1-3] and its possible relation to infectious mononucleosis,[4, 5] a study has been made of the response of different tissues to experimental infection. The alterations stimulated by organisms of this group both in the peripheral blood and in the visceral organs of several species of animals have been described by other workers, and their reports have been reviewed recently by Conway.[6] In this communication, however, attention has been drawn to the lesion occurring in the eye; first, because a detailed histological description of the ophthalmic reaction has not heretofore been available and, secondly, because the corneal response provided suitable material for a study of capillary formation in infected tissue, avascular under normal conditions.

MATERIALS AND METHODS

Ophthalmic infections were regularly induced in rabbits and guinea pigs by simple instillation of several drops of a broth culture (18 to 24 hours) of the organism into the conjunctival sac.[1-3] Monkeys (*Macaca rhesus*), which respond irregularly to the inoculation performed in this way, were infected in some instances by intraconjunctival or subconjunctival introduction of organisms. Since the ocular infection was never fatal, animals were sacrificed at successive stages in the course of the disease and the ocular structures were removed for study. Palpebral tissue and the bulbus oculi were fixed in acetic Zenker's fluid; paraffin sections were prepared in the usual way and stained with hematoxylin and eosin. Pannus formation was further studied in whole mounts of the cornea prepared by a modification of the Spalteholz method.[7]

In addition, visceral lesions of rabbits, guinea pigs and mice infected by intravenous or intraperitoneal inoculation were studied. Tissues were obtained from animals which, for the most part, died of the infection or were sacrificed by intravenous injection of air. Paraffin sections were prepared from spleen, lymph nodes, liver and lungs which, after fixation in acetic Zenker's fluid, were stained with hematoxylin and eosin.

* Conducted under a grant from The Commonwealth Fund.
Received for publication, January 12, 1942.

these authors as characteristic of infectious mononucleosis arise independently of infection with Listerella.

THE OPHTHALMIC REACTION

The ocular response of rabbits and guinea pigs to instillation of cultures of Listerella is essentially identical, and it is stimulated equally well by the different immunological types of the organism.[2, 3] Monkeys are more resistant to infection and display a milder reaction even when the bacteria are injected into the subcorneal tissue. Because of the similar response of different animal species, detailed discussion of the ophthalmic reaction will be limited to the observations made on infected rabbits.

Clinical Course of the Ophthalmic Reaction

The reaction was initiated by an intense conjunctivitis which appeared within 24 to 48 hours after the instillation of organisms and which was characterized by a thick, profuse discharge, chemosis, swelling of the lids and hyperemia of the palpebral and bulbar conjunctiva. A few days later the cornea also participated in the infection, exhibiting at first turbidity, then the development of capillaries, and eventually the formation of a heavy pannus which originated from the entire circumference and approached the center of the cornea. Figure 1 illustrates a typical involvement observed in the eye of a rabbit. Hypopyon, iritis and corneal ulceration have been encountered only exceptionally and, therefore, they should not be considered as characteristic of Listerella infection. The acute ophthalmic infection usually subsides in 2 to 3 weeks and complete clinical recovery occurs.

It may be pointed out that infection of the eye has not been encountered following inoculation by any route other than ocular. Conversely, generalization of the infection with septicemia and the development of lesions in the viscera have not been observed in animals with an ophthalmic reaction.

Daily blood counts were performed on a group of rabbits following ocular instillation of organisms in order to determine whether the hematological changes considered typical of infection with Listerella also occurred in this type of localized disease. Analysis of the data revealed no regular change in the quantity or quality of circulating white blood cells.

Anatomical Changes Observed in the Ophthalmic Reaction

Anatomical changes were studied in two types of material; *i.e.*, histological sections prepared in the usual manner, and corneal mounts prepared from eyes whose vessels had been injected with a colored

Results

Visceral lesions encountered in generalized infection

Small quantities of *Listerella monocytogenes* injected into rabbits, guinea pigs and mice by any of several routes initiated a generalized infection, commonly terminating in death within a few days. The predominant changes consisted of areas of focal necrosis in the liver, spleen and lungs. In some animals the areas of necrosis suggested massive abscesses, but in every instance there was present an appreciable number of mononuclear cells, an unusual finding in abscesses caused by the more common pyogenic bacteria. A similar type of response to an overwhelming infection with Listerella has been reported previously by Bloom.[8, 9] A much milder mononuclear reaction was observed following injection of smaller doses of the organism. In the liver and lungs, moreover, small accumulations of lymphocytes and monocytes were encountered in the periportal connective tissue and in the peribronchial tissue, respectively.

Conway [6] has studied exhaustively the development and regression of the lesions in the spleen and lymph nodes of rabbits and guinea pigs following infection with this organism. Since the present observations are confirmatory of her findings, they may be briefly summarized. Within the first 48 hours the size of the malpighian bodies of the spleen was greatly increased by lymphocytes, monocytes and transitional forms. Within a comparatively few hours the cellularity of the white pulp decreased, but the red pulp now contained a great number of mononuclear cells; by 120 hours the spleen had returned to normal. The lymph node behaved similarly and showed a corresponding increase and proliferation of lymphocytes and monocytes. This activity persisted for 96 hours, but at 120 hours the organ regained its normal appearance.

An effort was made to determine whether the so-called "infectious mononucleosis cell" which Gall and Stout [10] described in lymph nodes excised from patients, occurred in the lymph nodes of rabbits experimentally infected with Listerella. Nodes were removed at different stages of infection from the omentum and inguinal region and occasionally from the axillary area. Paraffin sections were stained with phloxine and methylene blue as recommended by Gall and Stout. Despite careful and repeated search the "infectious mononucleosis cell" was never encountered in any of the material studied. Furthermore, the general appearance of the nodes showed none of the structural modifications observed by these authors in infectious mononucleosis. It would seem more likely, therefore, that the changes considered by

thin and slender. Capillary loops of the variety seen earlier were still encountered but the connecting arches had disappeared from a number of vessels which now terminated as blind tubes. Many pin-point hemorrhages had spread into the interlamellar spaces from the distal ends of some of the vessels. These ecchymoses, however, were best seen by *in vivo* examination of the eye. As the infection progressed, looped vessels were seen to arise from corneal capillaries instead of from the conjunctival vessels. Capillaries were now observed not only connecting the vessels of the same level but also between vessels of adjoining levels. The offshoots of the conjunctival vessels were thicker at 5 days but they were not so numerous as earlier.

Intermediate Stage. Observations on the two types of preparation showed that the most severe lesions and the greatest development of the pannus occurred about 9 to 10 days after inoculation.

Microscopical sections revealed denser foci of mononuclear cells at the limbus, and polymorphonuclear infiltrations of the cornea were more intense than previously observed. In some instances a shallow ulcer was present on the corneal surface; it was characterized by complete loss of epithelium with a base formed by a collection of polymorphonuclear cells and débris. In other sections, the epithelial surface was intact but inflammatory lesions extended down through the cornea to the anterior chamber. The edema of the substantia propria was more marked. Mononuclear and polymorphonuclear leukocytes were observed throughout the substantia propria from the epithelium to the membrane of Descemet; the cells of the adjacent corneal endothelium appeared swollen and vacuolated.

The infection had spread down into the ciliary body; the iris was not invaded, but its vessels were dilated. In none of the sections was there evidence of an accumulation of cells or débris in the fluid of the anterior chamber. Structures of the posterior chamber showed no changes.

The epithelial cells of the bulbar conjunctiva were hydropic; the underlying collagenous tissue was edematous and lightly infiltrated by cells of the mononuclear and polymorphonuclear series.

About the twelfth day the intensity of the reaction began to diminish. There were fewer cells collected at the limbus, and there was only a slight infiltration of the cornea beyond this point. The polymorphonuclear leukocytes disappeared, leaving only foci of mononuclear cells. The vessels were now being resorbed.

Corneal mounts prepared from eyes removed on the ninth day clearly revealed the main vascular stems at the limbus. These were quickly lost in the dense capillary network which was characterized

mass before fixation. Microscopical examination under low magnification of the latter type of preparation assisted in correlating the vascular reaction with the cellular changes encountered during the infection. The various phases of the reaction may be roughly grouped as early, intermediate and late stages.

Early Stage. Microscopical sections from the eyes of rabbits as early as 2 days after instillation showed a moderate accumulation of cells around the marginal vessels which are normally present at the sclerocorneal junction. Small lymphocytes comprised the majority of the infiltrating cells, but a few monocytes and polymorphonuclear leukocytes also were present. As the process advanced, the accumulation of cells about the vessels at the limbus increased. The majority of the cells observed on the fourth day were still mononuclear in character, consisting of small lymphocytes, monocytes and occasional macrophages; there were, however, many polymorphonuclear cells present at this stage. The invading cells were distributed centripetally along the lamellae of the edematous substantia propria toward the center of the cornea. Away from the sclerocorneal junction, the cells progressively decreased in numbers and the infiltration changed in character so that polymorphonuclear cells replaced mononuclear cells a short distance from the limbus. New vessels arising from the marginal vascular loops extended into the cornea. The corneal epithelium was thickened in places, especially over the large foci at the limbus, and many of the epithelial cells were in mitosis. In one section, polymorphonuclear leukocytes appeared to have invaded not only the substantia propria but also the overlying flattened corneal epithelium; it was evident that an ulcer was in process of formation in this region. A few mononuclear cells had invaded the sclera close to the sclerocorneal junction. The connective tissue fibers of the bulbar conjunctiva were widely separated by edema, and there was a scattering of mononuclear cells under the epithelium.

The *corneal mount preparations* made on the second day after inoculation revealed the penetration of the cornea by capillaries arising from both the marginal and conjunctival vessels. At this time the newly formed vessels were barely detectable in the gross even with the aid of a hand slit lamp or loupe. Examined under a dissecting microscope, the capillaries appeared as tiny, delicate loops returning to their original source, thus completing the circulation. The loops varied in number and were usually seen at different levels in the cornea. By the fifth day the vessels had penetrated three or four times farther than on the second day. The caliber of the vessels now varied greatly; the older ones were thicker, while the more recent ones were

studied at this time, meningo-encephalitis has been reported in Listerella infections in man [14] and animals.[15]

Histopathological changes which develop in the eyes of rabbits and guinea pigs following instillation of Listerella organisms into the conjunctival sac have been studied. The early lesion consists of collections of mononuclear cells about the vessels of the limbus at the sclerocorneal junction. As the disease progresses, polymorphonuclear and mononuclear leukocytes invade the cornea and, to a lesser degree, spread into the sclera. The cornea becomes edematous and highly vascularized. Rarely, ulceration of the surface of the cornea occurs. The process reaches its peak about 9 days after infection, and then gradually recedes; the cornea generally returns to normal between the second and third week. Infection effected by instillation of Listerella on the uninjured cornea remains localized in the eye, and, conversely, parenteral injections of the organisms result in visceral lesions but never infect the eye.

Vascularization of the infected cornea is similar in most respects to the corresponding process observed in allergic inflammatory reactions,[7] or in trachomatous infection.[13] Vascular loops spring from the conjunctival and corneal vessels. These loops become separated at their centers, leaving stems from which blood extravasates. After several repetitions of loop formation and degeneration, the capillaries eventually complete the process of vascularization by throwing out crowfoot or arborescent terminations. The vascularization of the cornea is at its height about 10 days after infection and it requires 3 weeks or more for complete healing.

Summary

The histopathological changes in the ophthalmic response of several species of animals to infection with organisms of the genus Listerella have been studied. The ocular reaction after instillation of organisms is characterized by an inflammatory response in the conjunctiva and cornea without appreciable involvement in the deeper structures of the eye. In general, the cellular infiltration in the first few days consists principally of lymphocytes, but by the fifth day the proportion of polymorphonuclear leukocytes increases until these cells predominate by the tenth day. Edema of the involved structures is a prominent feature at the height of the infection. Vascularization of the cornea develops concomitantly with the other evidences of inflammatory response. The pathological changes rapidly recede and the eye generally heals without scarring 2 to 3 weeks after instillation of organisms.

by a tremendous degree of crowfooting at the terminus of most of the vessels. Arches and loops had almost entirely disappeared and the petechial hemorrhages in the corneal spaces were intensified. The capillary network began to recede at about the time when the cellular infiltration regressed. First, crowfooting disappeared and the capillaries appeared as long, slender vessels with a minimum of branching; then these decreased in number until only a few remained.

Late Stage. Microscopically, there was little evidence of the preceding infection 3 weeks after instillation of bacteria. At this time the cornea appeared to be almost normal; there were only small accumulations of mononuclear cells limited to the limbus; the recent vascular proliferation had disappeared; and the epithelium was now intact.

Corneal mounts likewise revealed a normal vascular bed in 3 to 5 weeks after infection.

Reaction of Periocular Structures. The palpebral conjunctiva, like the bulbar conjunctiva, showed the effect of the infection. During the first 5 days there was an infiltration of the epithelium and of the underlying tissue by polymorphonuclear and mononuclear cells. After this period the polymorphonuclear leukocytes disappeared, leaving a scattered collection of mononuclear cells. The connective tissue was edematous in the early stage. It is of interest to point out that healing of the conjunctiva by cicatrization has never been observed.

Discussion

The lesions encountered in this study in the spleen, liver and lung of rabbits, mice and guinea pigs infected with the genus Listerella are essentially identical with those described originally by Murray, Webb and Swann [11] and subsequently by other workers.[5, 9, 12, 13] In general, these consist of areas of focal necrosis scattered through the organs. When overwhelming doses of bacteria are injected, however, massive necrotic areas develop which differ from ordinary pyogenic abscesses only in the presence of a preponderance of mononuclear leukocytes.[8, 9] In the lymphoid tissue of the spleen an increase in the size of the malpighian bodies due to the proliferation of the mononuclear elements occurs first; then the bodies decrease in size and the mononuclear cells appear in the red pulp. A similar cycle is also observed in the lymph nodes.[6, 8, 9] In the present study of the lymph nodes there was no evidence of the "infectious mononucleosis cell" which Gall and Stout [10] described in lymph nodes taken from humans suffering from infectious mononucleosis. While histological observations on the central nervous system were not included in the animals



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Julianelle and Moore Pathological Changes in Listerella Infections

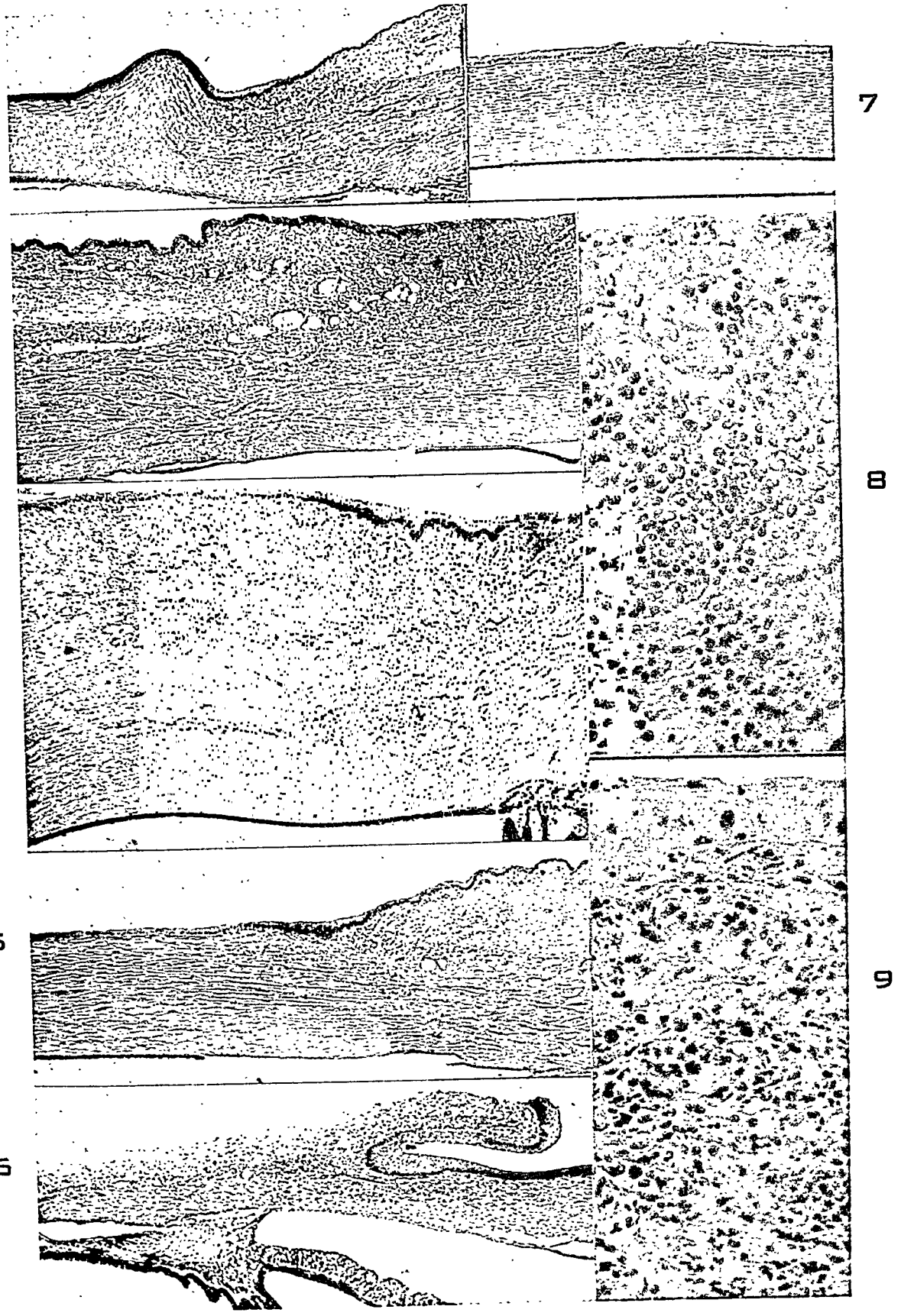
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DESCRIPTION OF PLATES

PLATE 119

FIG. 1. Clinical appearance of rabbit eye 7 days following instillation of Listerella. Thick exudate, edematous and inflamed lids, and corneal reaction of marked clouding and massive vascularization are shown. × 3.



Julianelle and Moore Pathological Changes in Listerella Infections

PLATE 120

FIG. 2. Cornea of rabbit 2 days after instillation of Listerella organisms. A small collection of cells is present around the marginal vessels of the sclerocorneal junction. Higher magnification shows these to be principally lymphocytes. Hematoxylin and eosin stain. × 38.

FIG. 3. Cornea of rabbit 5 days after infection. A marked increase in the cellular infiltration and in vascularization has occurred since the stage illustrated in Figure 1. Lymphocytes and mononuclear leukocytes still make up the bulk of the infiltrative reaction but polymorphonuclear leukocytes are beginning to appear. The lamellae of the cornea are spread apart by edema. Hematoxylin and eosin stain. × 38.

FIG. 4. Cornea of rabbit 9 days after infection. The accumulation of cells at the limbus is still dense and the infiltration has extended toward the center of the cornea. Polymorphonuclear leukocytes form the majority of the inflammatory cells. Many newly formed vessels accompanied by infiltrating cells occur throughout the width of the cornea. The width of the corneal substantia propria has increased greatly due to edema. Hematoxylin and eosin stain. × 38.

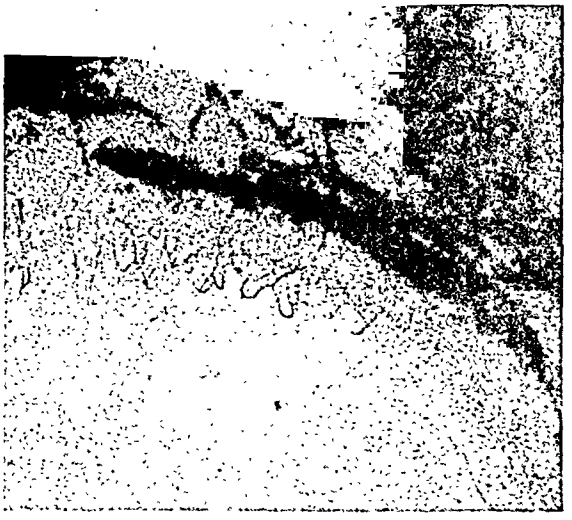
FIG. 5. Cornea of rabbit 21 days after infection. Only a slight collection of cells, principally lymphocytes, remains at the limbus. Most of the new vessels have disappeared. Hematoxylin and eosin stain. × 38.

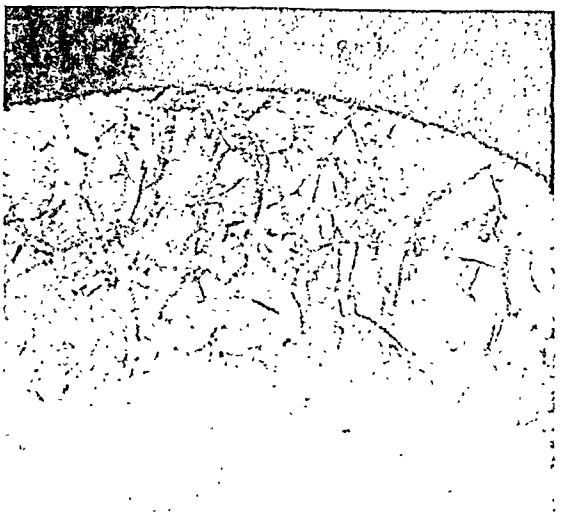
FIG. 6. Cornea of guinea pig 5 days after infection. The reaction is similar to that observed in the rabbit. Hematoxylin and eosin stain. × 38.

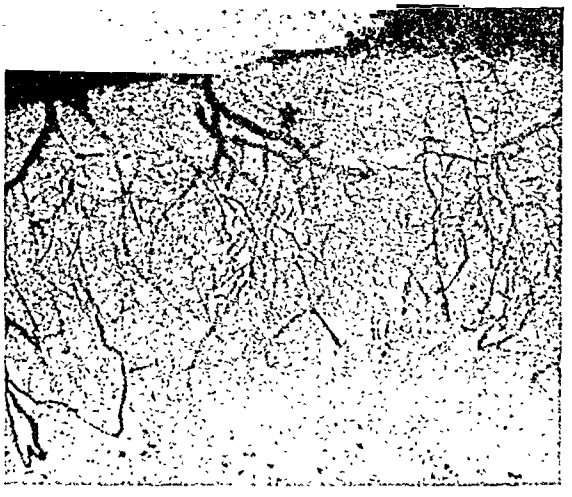
FIG. 7. Cornea of rabbit 21 days after infection. A small, shallow ulcer is apparent. Hematoxylin and eosin stain. × 38.

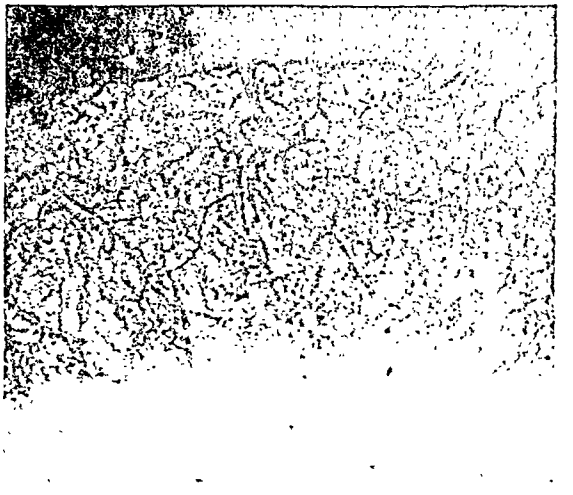
FIG. 8. Cornea of rabbit 5 days after infection. Lymphocytes and monocytes are clustered about small vessels. Hematoxylin and eosin stain. × 300.

FIG. 9. Cornea of rabbit 9 days after infection. The increased vascularity is apparent. The majority of the infiltrating cells are polymorphonuclear leukocytes. Hematoxylin and eosin stain. × 300.

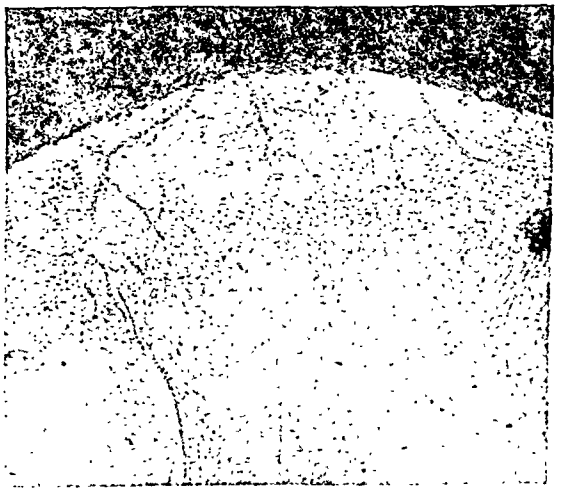












Julianelle and Moore

Pathological Changes in Listerella Infections

Plate 121

Figs. 10–15. Progress of corneal vascularization in the rabbit as seen in corneal mounts. Figures 10 and 11 present the changes at 2 and 5 days, Figures 12 and 13, at 8 and 10 days and Figures 14 and 15 at 12 and 18 days following instillation, respectively.

THE CHORIOALLANTOIC MEMBRANE OF CHICK EMBRYOS AND ITS RESPONSE TO INOCULATION WITH SOME MYCOBACTERIA*

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The great value of inoculation of the chorioallantoic membrane of the developing chick since it was first described by Levaditi[1] in 1906, when he studied the spirillum of fowls, has been adequately proven and enhanced by Goodpasture and his associates. The successful work done by numerous workers with such disease incitants as viruses, Rickettsiae, bacteria, the spirochetes and fungi has stimulated the undertaking of further investigation, utilizing the chorioallantois.

The infected membrane lends itself easily and within a short time to a careful study of the histopathogenesis of the disease process. The possibility of day by day or even hour by hour examination of the lesion facilitates the tracing, microscopically, of the various types of cells that may become involved in the evolution of such a lesion. In like fashion, the method has made it a relatively simple matter to follow the fate of the organism and to note changes in morphology, especially of fungi.[2]

A group of microorganisms known as Mycobacteriaceae and often referred to as mycobacteria or *acid-fast organisms* is thought by some to be closely related to the fungi. As a consequence of the experiments on the inoculation of the chorioallantoic membrane of chick embryos with fungi, similar work was begun with several strains of mycobacteria for several reasons. In the first place, lesions produced by several of the acid-fast organisms are often confused with various mycotic granulomata. It was thought that this procedure might shed light on their differentiation. Secondly, as with fungi, the evolution of an experimental lesion, particularly of tuberculosis or leprosy—mycobacterial diseases—is exceedingly long, requiring from weeks to months. It was hoped, therefore, that the length of time required to produce an experimental lesion might be shortened to a few days by the use of the chorioallantois. Thirdly, it has been the desire of various workers in tuberculosis to find a means of determining virulence in a relatively short time by a simple method; and fourthly, it was thought that the use of this membrane might also make it possible to

* Work done under a grant from the Medical Research Committee of the National Tuberculosis Association.

Received for publication, January 21, 1942.

from Dr. H. J. Corper; V. E. strain from U. S. Department of Agriculture; strain no. 36 from Dr. Florence Seibert, Henry Phipps Institute.

4. *Mycobacterium marinum* (fish). Strains nos. 1 and 2 from Dr. J. D. Aronson, Henry Phipps Institute.

5. *Mycobacterium thamnopheos* (snake). Strains nos. 1 and 2 from Dr. J. D. Aronson, Henry Phipps Institute.

6. *Mycobacterium leprae muris* (rat). Rats with lepromata obtained from Dr. E. V. Cowdry, Department of Anatomy, Washington University School of Medicine. Bacilli were expressed from lepromata. The lepromata varied in age from 8 to 10 months.

Eggs were incubated 12 days and then inoculated. Eggs incubated 10 days were found to be less hardy than those incubated 12 days and, therefore, were not used in subsequent series. In some series eggs were incubated 13 days. These served the purpose equally as well, but did not allow as much time for the development of lesions.

The inoculations in several series consisted of a loopful of organisms implanted on the ectodermal surface of the chorioallantoic membrane. In other series, amounts of 0.1 mg. to 1.0 mg. of bacilli were planted on the membrane. Best results were obtained with mass implantations and with the higher concentrations of bacilli.

Following inoculation, the eggs were incubated in a bacteriologic incubator. After 5 days in some series and after 6 days in other series, the inoculated membranes were removed, fixed in Zenker's solution (with 5 per cent acetic acid), embedded in paraffin and sectioned. The sections were stained for acid-fast organisms and duplicates stained with hematoxylin and eosin. These slides were studied to determine the character of the lesions.

Experimental Data

The experimental results obtained are encouraging and point to a number of interesting and distinguishing features which characterize the particular organism involved.

Mycobacterium tuberculosis hominis

Eggs were incubated 12 or 13 days and fixed 5 or 6 days after inoculation. Many of the embryos died. A small number in each series remained alive, but apparently were considerably weakened. These showed nodules on the membrane with infiltration and thickening of the membrane. The nodules were either discrete or confluent, with a peripheral opacity of light grayish infiltrate.

Interspersed among the bacilli of the inoculum were seen a large

differentiate the various strains of mycobacteria by the type of reaction produced in the inoculated chorioallantois.

The published reports dealing with the inoculation of the chorioallantois with mycobacteria are few in number. Goodpasture and Anderson,[3] in 1937, obtained lesions with *Mycobacterium tuberculosis avium*. The lesions manifested themselves within the membrane up to the fourth day; then as nodules of the drying chorioallantois. Costil and Bloch,[4] in 1938, were also able to obtain nodular growths within 7 days using both human and avian strains of tubercle bacilli. In a preliminary report, I[5] described experimental inoculations of the chorioallantois with human and avian tubercle bacilli and with leprosy bacilli. This was followed[6] by a report which included the additional strains of bovine, fish and snake tubercle bacilli. The final paper to date, as far as is known, is by Emmart and Smith[7] who utilized the chick membrane for inoculation with three strains of human and one strain of bovine tubercle bacilli.

Materials and Technic

The technic of inoculation of the chorioallantoic membrane of the developing chick, generally considered as the Goodpasture-Buddingh technic, is an elaboration by Goodpasture of a technic described by Clark.[8] The technic was adapted by Woodruff and Goodpasture[9] to obtain lesions with fowlpox virus. Goodpasture and Buddingh[10] further developed the experimental procedure to its present-day method of practicability. As used here, the technic is essentially the same as that described by them and as followed in other publications.[4,6,11]

Various strains of acid-fast organisms from different sources, some of known virulence, others not well determined, have been used in several series, some over a period of approximately 2 years. Several hundred eggs were utilized. These organisms were the following:

1. *Mycobacterium tuberculosis hominis*. Virulent strain from Department of Bacteriology, Washington University School of Medicine; strain H160 from Dr. H. J. Corper; PN strain from U. S. Department of Agriculture; strain A 18 from Dr. Florence Seibert, Henry Phipps Institute; virulent strain from Department of Bacteriology, St. Louis University Medical School; and nonclassified strains from St. Louis City Health Laboratories.

2. *Mycobacterium tuberculosis bovis*. Virulent strain from Dr. H. J. Corper; strain 854 from U. S. Department of Agriculture; bovine "S" strain from Dr. Florence Seibert, Henry Phipps Institute.

3. *Mycobacterium tuberculosis avium*. Strain from Department of Bacteriology, Washington University School of Medicine; strain 14,619

irregular giant cells with the nuclei scattered particularly around the periphery, with an opaque central area (Fig. 6). As the tubercle became older, the mononuclear cells extended outward from the central mass (palisading) and formed the giant cells which were characteristic of these tubercles.

There was marked hypertrophy of the ectoderm which manifested itself in the form of extensions into the mesoderm. These could be seen usually as "pearls," either detached from the ectodermal layer or, as is most likely, only apparently detached due to sectioning, whereby cross section made them appear as balls of epithelial cells in pearl formation. Occasionally the twisted connection could be seen clearly. Tubercle bacilli were found in the core of these "pearls" (Fig. 7). This indication of hyperkeratinization was not particularly marked, however, in the membranes inoculated with human tubercle bacilli.

The entoderm seemed little affected, if at all.

The significant points can be considered briefly as marked invasion of the mesoderm with virulent organisms; definite tubercle formation with perhaps caseation and necrosis to form "soft" tubercles; marked infiltration of the mesoderm with epithelioid cells, fibroblasts, monocytes and eosinophil-like cells accompanied by bacilli which may be found isolated, or in clumps, surrounded by various cells or engulfed by macrophages or giant cells of the Langhans type.

With less virulent bacilli the significant points are that the organisms do not invade the mesoderm rapidly; the mesodermal reaction is mild with few epithelioid cells, fibroblasts, monocytes, or giant cells; the organisms may appear freely distributed or they may be engulfed by macrophages or by giant cells of the Langhans type with little or no accompanying cellular reaction.

Mycobacterium tuberculosis bovis

Eggs were incubated 12 or 13 days and observed 5 or 6 days after inoculation. Many of the embryos died. The membranes of the living embryos showed, grossly, discrete or confluent plaques and nodules with an opacity of the membrane. The membrane appeared thickened in the area of marked involvement.

The first evidence as a result of inoculation with bovine tubercle bacilli was a nodular thickening of the ectoderm. The marked ectodermal hyperplasia resulted in a heaping up of the ectodermal layer with cornification or stratification of the outermost area. This reaction simulated parakeratosis in some areas while in others the appearance was that of hypertrophy and hyperkeratinization. As further evidence of this reaction, the ectodermal invagination into the mesoderm was

number of monocytes and leukocytes, the latter usually degenerating. At the margin of the inoculum was a layer of giant cells of the Langhans type. The ectoderm in this area underlying the inoculum seemed to have lost its normal character and was replaced by monocytes, degenerate polymorphonuclear leukocytes and the Langhans type of giant cells. In other areas, the hyperplastic ectoderm showed marked thickening, with the entire surface appearing flattened or in the process of desquamation.

In the mesoderm the reaction varied according to the virulence of the organism. With organisms showing decreased virulence the process simulated a mild foreign body reaction with very little cellular response except for a few mononuclears and some leukocytes which surrounded the bacilli. In other cases, bacilli were engulfed by a small giant cell, with very little other cellular response. Virulent organisms rapidly invaded the mesoderm and formed definite tubercles showing caseation necrosis. The mesoderm showed marked enlargement or swelling which was particularly apparent where the organisms had invaded the layer (Fig. 1).

The development of tubercles with virulent organisms seemed to follow a pattern or sequence of events. In the first place, organisms planted on the ectoderm either invaded the mesoderm as single cells which multiplied to form clumps or bundles of parallel cells, usually rods, with some showing beaded forms (Fig. 2); or, and this seemed to be the customary method, they were taken up by wandering cells (clasmatocytes, phagocytes) of the ectoderm and carried into the mesoderm. Secondly, the wandering cells lodged in the mesoderm and the bacilli multiplied, causing the host cells to become swollen and then to rupture, liberating the organisms. The invasion by the bacilli was followed by a rapid formation or calling forth of mononuclear or epithelioid cells and monocytes to the site of the bacilli, which in time was followed by a marked fibroblastic response, interspersed with some eosinophil-like cells (Fig. 3). The evidence of rapidity of cellular response was indicated by the large number of mitoses of the epithelioid cells which were seen in the area of the organisms (Fig. 4). Just as there was an increase in the number of epithelioid cells and fibroblasts, so was there likewise an increase in the number of capillaries. The capillaries were most numerous in the area of greatest activity. The capillary walls seemed thinner and eventually ruptured and formed the central area of necrosis and perhaps caseation. The resulting tubercle was of the "soft" type (Fig. 5).

Early tubercles showed an accumulation of mononuclear cells engulfing bacilli. The mononuclear cells apparently fused, producing

The reactions obtained with avian tubercle bacilli varied according to the virulence of the strain of organism used. The differences in membranal response, however, were sufficiently characteristic to make differentiation possible.

Grossly, the response of the membranes to virulent avian tubercle bacilli have already been described by Goodpasture and Anderson.[3] The membranes first showed a thickening, becoming clear and gelatinous, with the lesions apparently within the membrane. After about 5 days the membranes became dry and showed nodular formations. Microscopically, the finding of organisms in foci within the mesoderm confirmed this observation (Fig. 12).

Organisms planted on the membrane were rapidly taken up by mononuclears or phagocytes, perhaps clasmatocytes, and rapidly penetrated into the mesoderm where they set up foci. In these membranes, the ectoderm showed little or no inflammatory response, except that some bacilli were seen in ectodermal cells. The mesodermal response was not outstanding except for a proliferation of fibroblastic cells, within some of which bacilli were found. The number of bacilli in the macrophages or in the fibroblasts varied. In some there were a few rods while in others the host cell was packed (Fig. 13). In the mesoderm, no extracellular bacilli could be noted and this simulated closely the reaction seen in leprosy where the lepra bacilli are taken up by macrophages termed "foam cells." The bacilli were chiefly of the rod type, but coccoid forms were also seen and these were all acid-fast. Giant cells have not been found where virulent organisms were inoculated, but some epithelioid cells were apparent.

Avian bacilli that were somewhat less virulent produced a slightly different picture. The ectoderm became involved where the bacilli were planted. There was an ectodermal response with some hyperplasia and resultant inflammation. The bacilli were taken up by macrophages. The area was infiltrated with monocytes and epithelioid cells and red blood cells could also be seen. The macrophages loaded with bacilli penetrated into the mesoderm and occurred in clumps or were seen at a distance from the main group of loaded phagocytes (Fig. 14). The number of capillaries was increased in the areas of greatest response. Some capillaries were surrounded by monocytes, although the reaction was not intense. Certain large capillaries as well as other large blood vessels showed, in addition to numerous red blood cells, groups of monocytes. Fibroblasts were seen in large numbers but these were found chiefly in zones at the periphery of the lesion. Giant cells were not seen in any number in the mesoderm; however, small giant cells of the Langhans type could be seen at the base of the inocu-

particularly well noticed in the formation of large numbers of "pearls," formed especially in the mesodermal area just underlying the affected ectoderm and extending through the mesoderm (Fig. 8).

In other areas of the membrane, the ectoderm appeared to have been separated from the rest of the membrane, as though by fluid. In still other areas, especially where there was marked reaction, the ectoderm was replaced by many monocytes, polymorphonuclear leukocytes, epithelioid cells, some basophilic leukocytes and blood cells.

Invasion of the mesoderm by the bacilli was more apparent with a virulent strain. The number of organisms found in the mesoderm was in no case as great as in the same layer in membranes inoculated with virulent human tubercle bacilli. The mesodermal response, although made apparent by the presence of epithelial "pearls," showed little other activity except for swelling in affected areas and for the formation of scattered early tubercles (Fig. 9). These tubercles differed somewhat from those seen with inoculations of human strains. They were made up essentially of mononuclear or epithelioid cells, some fibroblasts, leukocytes and giant cells (Fig. 10). In some areas showing bacilli, "pearls" were found which contained organisms apparently from the ectodermal inoculum (Fig. 11). The presence of bacilli in these structures would seem to make them macrophages, no doubt of epithelial origin. Scattered freely among these cells or engulfed by the macrophages were the bacilli, which appeared as rods, either singly or in clumps, chiefly the latter. Capillaries seemed increased in number, extending particularly to the ectodermal surface and at the upper portion of the mesoderm at the epidermal junction. Capillaries also seemed more abundant in the vicinity of the tubercles. Necrosis was slight or lacking, and in no case marked. Caseation was not noticed in any of the tubercles seen.

The entoderm showed little response to the invading bacilli.

Tubercle formation as found in membranes inoculated with bovine tubercle bacilli is, therefore, quite different from that produced by human tubercle bacilli in that the tubercles lack caseation and necrosis is slight or lacking; epithelial hyperplasia is more apparent, manifesting itself as marked ectodermal proliferation into the mesoderm in the form of ectodermal "pearls" as seen on cross section.

Mycobacterium tuberculosis avium

Eggs were incubated 12 days and observed 5 days after inoculation. A number of eggs were examined on the third and fourth day after inoculation. The embryos became weak on the third day, with several dying on the fourth day and most of them dying on the fifth day.

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there was marked reaction, the ectoderm was either necrotic or ulcerating with an infiltrate of many monocytes, or it was separated from the mesoderm. Hyperplasia of the ectoderm was further emphasized by the formation of epithelial "pearls" which extended into the mesoderm. In the cores of some of these were found *Mycobacterium marinum* (Fig. 16).

Where the mesodermal reaction was most marked, the membrane was greatly thickened. In this case the rapidly developing granulomatous reaction presented several outstanding characteristics which easily separated the lesion from that caused by either human, bovine or avian tubercle bacilli. In the first place, the number of polymorphonuclear leukocytes was surprisingly great. These cells were seen scattered through the affected mesoderm, either singly or in clumps or groups forming microscopic abscesses. There were also basophilic leukocytes in varying numbers. Monocytes were also seen throughout the membrane and these, too, were found in clusters.

Another outstanding phenomenon was the presence of many groups of epithelioid cells, monocytes, fibroblasts and polymorphonuclear leukocytes, interspersed among which were bacilli occurring either singly or in clumps (Fig. 17). In the center of these groups there were capillaries which were apparently affected by the reaction since many showed broken walls and an infiltrate of monocytes and leukocytes. The capillaries throughout the affected area were greatly increased. Giant cells were not seen, but the groups of cells undoubtedly represented tubercle formation.

Acid-fast organisms were found chiefly as long rods, beaded, occurring freely, singly, or in clumps and usually within the group of cells.

Serial passage of ground-up infected membrane from egg to egg showed on the fourth passage a rapid invasion of the mesoderm with an accompanying monocytic and leukocytic infiltrate. The organisms apparently multiplied rapidly since they were seen in large clumps. The ectodermal reaction was likewise noticeable, but did not show marked hyperplasia. The reaction apparently was chiefly in the mesoderm (Fig. 18).

The entoderm appeared to be not affected.

The salient features of membranes inoculated with *M. marinum* are, briefly, a rapid invasion of the membrane, particularly the mesoderm; a granulomatous response with the presence of many polymorphonuclear leukocytes, monocytes and epithelioid cells; marked reaction involving the capillaries; the occurrence of many acid-fast organisms, either singly or in clumps, and no evidence of giant cell formation.

lum on the ectoderm. These formed a layer and were made up apparently of groups of mononuclear cells with the nuclei arranged peripherally, acting perhaps as foreign body giant cells (Fig. 15).

Where the organisms had penetrated in large clumps, no doubt part of the inoculum, with many bacilli embedded in an opaque substance, there was also a heavy infiltrate of monocytes, leukocytes and epithelioid cells. In this case, the clumps of organisms in the opaque matrix were surrounded by epithelioid cells. This induced the formation of giant cells of the Langhans type and was, therefore, a foreign body reaction, in which the foreign substance was the clump, with the epithelioid or mononuclear cells fusing to form large macrophages. Here there was also an increase in capillaries and polymorphonuclear leukocytes. Fibroblasts were also seen at the periphery of the mesodermal involvement, as well as basophilic leukocytes. The whole appearance was that of a foreign body granuloma. The entoderm was not affected.

With mild or avirulent organisms the reaction was almost entirely limited to the ectodermal implant with a marked infiltrate of monocytes and leukocytes. Invasion of the mesoderm by bacilli was mild and the mesodermal reaction was also mild except for some monocytes.

The chief points to be considered in chorioallantoic inoculation with avian tubercle bacilli are, first, the fact that the bacilli are taken up by macrophages showing intracellular bacillary growth with no extracellular bacilli in the invaded mesoderm. The reaction of the mesodermal layer to this invasion is not remarkable, showing many monocytes, leukocytes, epithelioid cells and an increase in number of capillaries. Giant cell formation is chiefly a foreign body reaction and is not as noticeable in these tubercles as in those formed as a result of inoculations with human tubercle bacilli.

Mycobacterium marinum

Eggs were incubated 12 days and observed 6 days after inoculation. Many of the embryos were dead, but grossly the membranes of the live embryos showed discrete or confluent gray plaques. The membranes seemed thickened and somewhat gelatinous in appearance. The reaction appeared to be within the thickened membrane to a greater extent than on the surface. However, there were a number of small, discrete plaques which were seen as small, raised nodules on the membrane.

Microscopically the fish tubercle bacilli produced a striking picture of rapid invasion and granulomatous response. Where the organisms had come in contact with the ectoderm to form nodules, the ectoderm was hyperplastic, thickened and raised, showing a parakeratotic type of response as well as keratinization. In other areas, particularly where

opaque to grayish yellow areas, plaques and nodules. The general appearance indicated an inflammatory response of the membrane.

Microscopically, the ectoderm, in the area inoculated with organisms expressed from rat lepromata, was apparently disintegrated and invaded by many monocytes and macrophages or epithelioid cells. The inoculum with the resulting reaction made the lesion appear nodular, which may be compared with a nodular leproma (Fig. 22). At the margins of the lesion the ectoderm was thickened, with some hyperplasia, as evidenced by the presence of "pearls" in the adjacent mesoderm (Fig. 23).

At the junction of the inoculum and the affected mesoderm could be seen giant cells made up of epithelioid cells within which there were many bacilli. Fibroblasts extended along this area and appeared to be penetrating the inoculum. Capillaries were also found in larger numbers along the upper portion of the mesoderm, with many present in the deeper mesoderm. Polymorphonuclear leukocytes were also seen in large numbers. All of these cells were more numerous in the area underlying the inoculated ectoderm.

Of chief importance was the presence of large numbers of large phagocytes or macrophages with an eccentric nucleus. These cells, when stained with hematoxylin and eosin, appeared somewhat vacuolated (Fig. 24). When colored with carbol fuchsin they were found to be loaded with lepra bacilli. Stained in this manner, many of these phagocytizing cells showed large vacuoles, with the organisms filling the cell and causing it to become swollen. Formations of various types were seen in these cells. Semilunar arrangements and the formations termed globi were also made out. These "lepra" or "foam" cells are actually phagocytes (Fig. 25).

A striking resemblance is seen between inoculations of avian tubercle bacilli and those of lepra bacilli on the chorioallantoic membranes. The bacilli in both cases are chiefly intracellular. The chief difference, however, is in the type of phagocytizing cell. In the former the phagocyte is an epithelioid cell; in the latter it appears to be a specialized "lepra" cell. A nodular reaction is another important feature of membranes inoculated with *Mycobacterium leprae muris*.

Discussion

The inoculation of the chorioallantoic membrane of the developing chick with acid-fast bacilli is a new approach to the study and determination of the rôle played by these organisms in infectious processes. The relative simplicity of the technic lends itself easily to its application in experimental pathologic problems. But what is more important

Mycobacterium thamnopheos

Eggs were incubated 12 days and observed 6 days after inoculation. Some of the embryos died, but a number were alive.

Grossly, the membranes showed grayish nodules or plaques which were spread over the membrane. The membrane was thickened in these areas and showed inflammation.

Microscopically, there were marked inflammatory changes in the ectoderm as well as in the mesoderm. The ectoderm was irregular, necrotic and infiltrated with monocytes and leukocytes. The appearance was that of an ulcer. Where the ectoderm was not destroyed, it was thickened, and showed an inflammatory reaction.

The presence of giant cells was a remarkable feature in these membranes. These were formed in the ectodermal reaction at the base of the inoculum, many fusing to form tubercles. They were seen in large numbers and extended into the mesoderm where they also fused to form tubercles (Fig. 19). The cellular reaction was notably of fibroblasts. These were seen in large numbers, but there were also some basophilic leukocytes and mononuclears. The number of capillaries was greatly increased. There was a marked reaction around the capillaries with many fibroblasts and polymorphonuclear leukocytes. A number of the capillaries had apparently ruptured since red blood cells could be seen scattered throughout the mesoderm.

Mycobacterium thamnopheos is difficult to stain in sections; consequently it was not possible to determine how far the organism had penetrated into the mesoderm. However, the marked response of the mesoderm and the finding of giant cells and groups of inflammatory cells deep in this layer would seem to indicate that the organism had been able to cause damage deep in the mesoderm where it no doubt invaded (Figs. 20 and 21).

The entoderm showed some hyperplasia in the area underlying the marked mesodermal and ectodermal response.

Of chief interest in membranes inoculated with *M. thamnopheos* is the great number of giant cells, many of which have fused to form tubercles, some showing caseation. The fibroblastic response is remarkable as is the activity in and around the capillaries.

Mycobacterium leprae muris

Eggs were incubated 12 and 13 days and observed 6 days after inoculation. Most of the embryos died, with the remaining live ones extremely weakened.

Macroscopically, the membranes appeared thickened, showing

This work makes possible the investigation of the action of drugs or chemical fractions of organisms in inhibiting or enhancing the growth of the bacilli and thus determining the value of such agents in combating the disease.

Summary

1. Strains of human, bovine, avian, fish and snake tubercle bacilli and of rat leprosy bacilli have been inoculated in the chorioallantoic membrane of the developing chick.

2. The histopathogenesis of the lesion produced by these various acid-fast organisms has been studied and differentiated.

3. The type of reaction and the degree of response of the membrane to the inoculum serve to determine virulence in a relatively short time.

4. The possibility of further work on methods of study, including the action of drugs or chemical fractions of the bacilli, are suggested.

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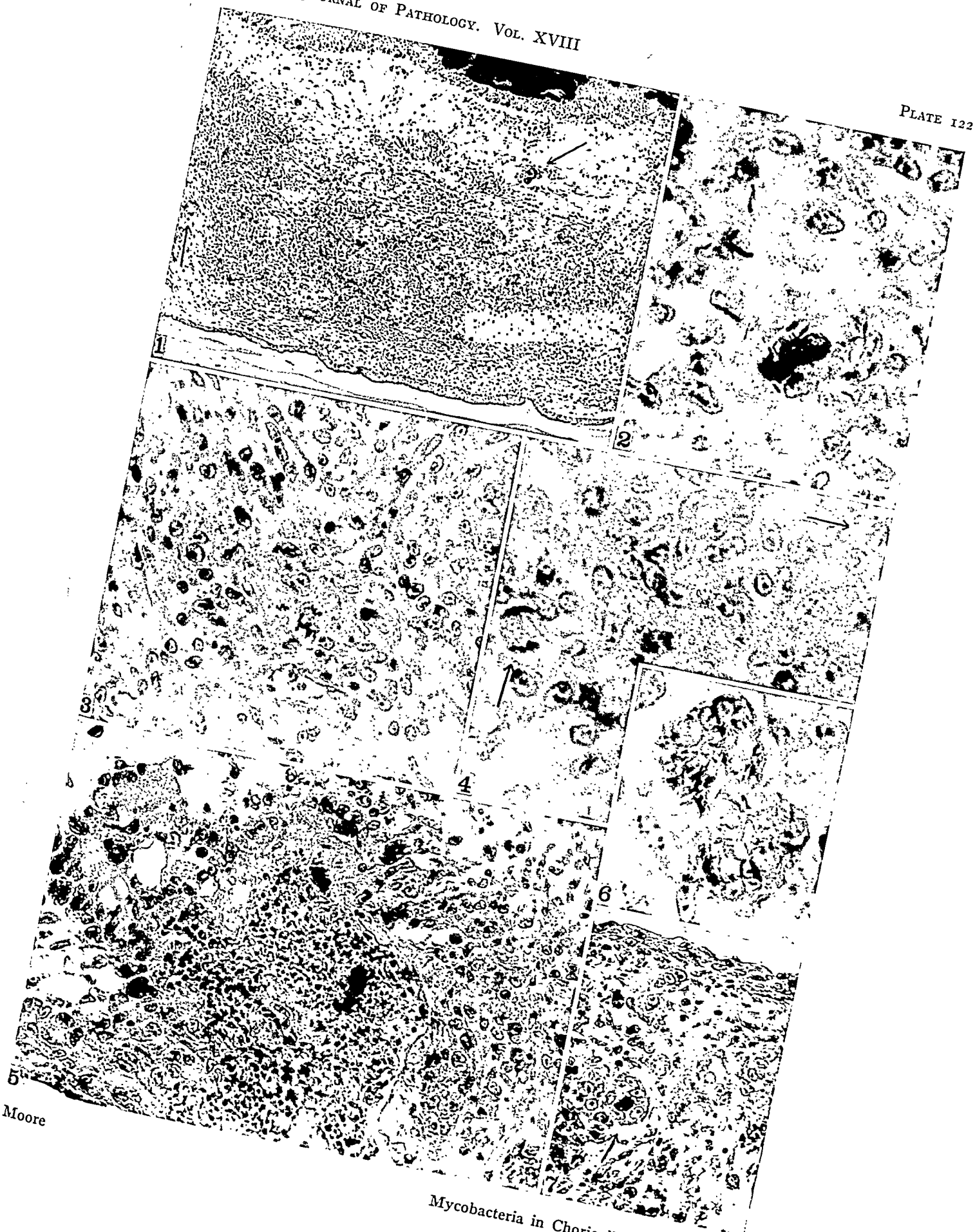
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is the well controlled test-subject as used in the procedure. The fertilized egg is easily controlled, both as to strain and temperature conditions. Food supply is eliminated as a factor. The possibility of existing or pre-existing infections can be ruled out as complicating the picture produced by the inoculum. Of equally great importance is the low cost per egg as compared with the cost of laboratory animals.

The study described here is concerned chiefly with the results when live mycobacteria are inoculated in the chorioallantois. Several important points have been described. In the first place, the histopathogenesis of these acid-fast bacterial diseases was easily traced. By this study the type of reaction produced by the various strains of bacilli could be differentiated. Certainly, virulence can be determined without difficulty. The rapid formation of tubercles with virulent strains definitely distinguishes these organisms from the nonvirulent or less virulent strains where tubercles are either of slow development and few, or lacking. The concerted action on the ectoderm in the inoculations of the bovine strain as compared with the mesodermal response in the membranes inoculated with human strains is an important differential point. The exclusively intracellular development of avian tubercle bacilli, their rapid spread into the mesoderm as seen in virulent strain inoculations and the different type of cellular response help easily to distinguish this organism from both human and bovine strains.

The rapidity in the formation of tubercles with virulent strains of tubercle bacilli in the short space of 5 or 6 days and perhaps in less time, when compared with the weeks or months necessary for the formation of tubercles in laboratory animals, favors the egg technic. These are only a few of the advantages. What perhaps appeals greatly to the technician is the finding of a method for the rapid identification of strains of tubercle bacilli and for the determination of their virulence. This can be done in the chorioallantois since the type of reaction and the intensity of the response are adequate for this purpose.

The results of inoculation of fish and snake tubercle bacilli further emphasize the value of the chorioallantois, for in them we have organisms occurring in cold-blooded animals which are able to produce definite lesions in a warm-blooded test-subject. This certainly opens the path for further experimental work with these acid-fast organisms. Again, the inoculation of the rat leprosy bacillus and the resultant lesion and response present a microscopic picture so characteristic of the spontaneous lesion that here, too, the path to further investigation—both with rat and human leprosy—is cleared of a number of obstacles. One of the chief problems of investigative work is the element of time. With the egg membrane this is cut down to an almost insignificant factor.



Moore

Mycobacteria in Chorioallantoic Membrane

DESCRIPTION OF PLATES

PLATE 122

FIG. 1. Section through membrane inoculated with *Mycobacterium tuberculosis hominis*. Marked infiltration of mesoderm, with tubercle formation. Acid-fast stain. × 85.

FIG. 2. Group of bacilli in center of early tubercle. *Mycobacterium tuberculosis hominis*. Acid-fast stain. × 1025.

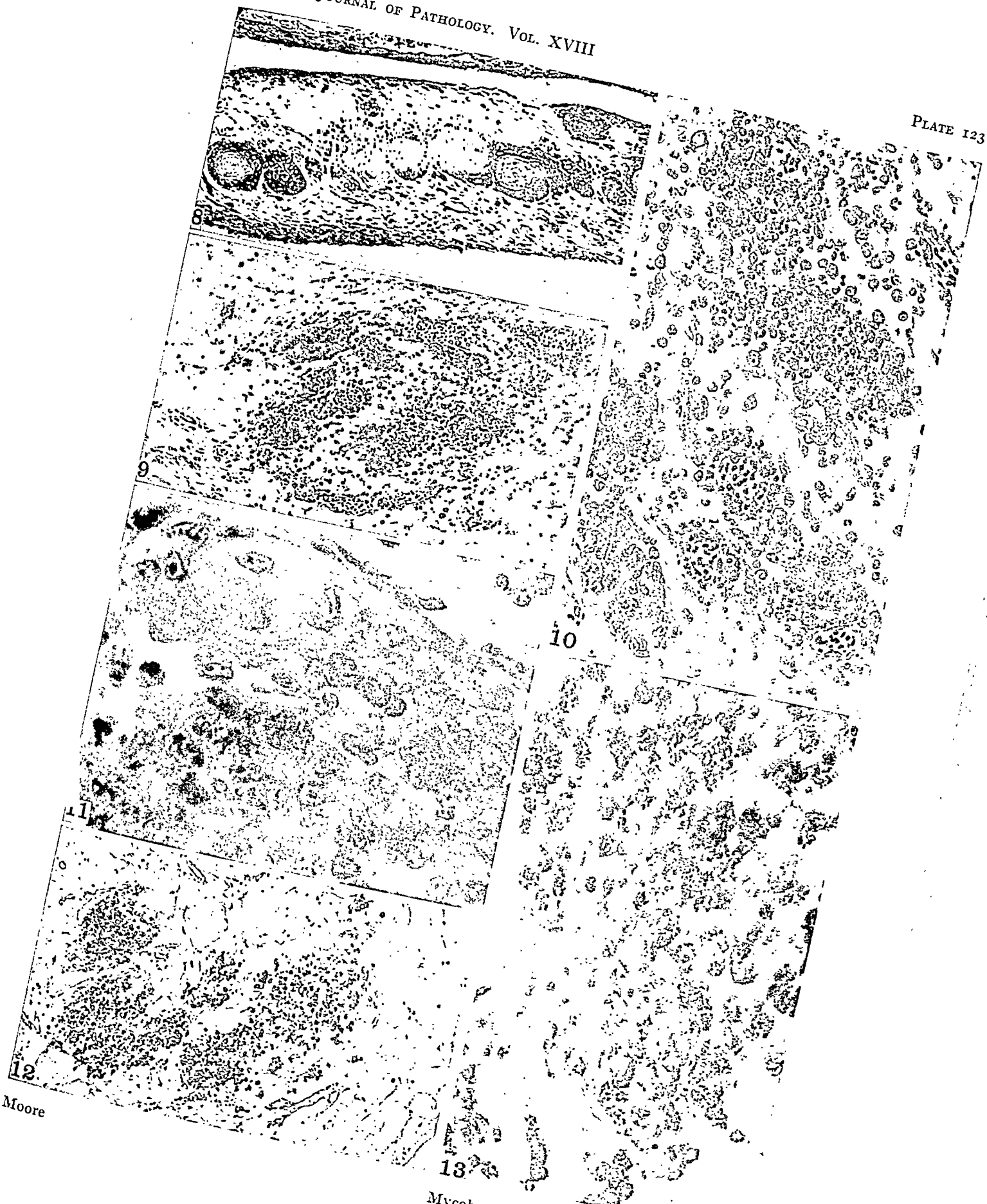
FIG. 3. Center of early tubercle formation. *Mycobacterium tuberculosis hominis*. Acid-fast stain. × 525.

FIG. 4. Mitotic cells in area of response to bacilli. *Mycobacterium tuberculosis hominis*. Acid-fast stain. × 920.

FIG. 5. Tubercle formation. Bacilli and a central area of necrosis are seen. *Mycobacterium tuberculosis hominis*. Acid-fast stain. × 420.

FIG. 6. Engulfed bacilli of *Mycobacterium tuberculosis hominis*. Acid-fast stain. × 900.

FIG. 7. *Mycobacterium tuberculosis hominis* in core of epithelial "pearl." Acid-fast stain. × 365.



Moore

Mycobacteria in Chorioallantoic Membrane

PLATE 123

FIG. 8. "Pearl" formation in membrane inoculated with *Mycobacterium tuberculosis bovis*. Acid-fast stain. × 110.

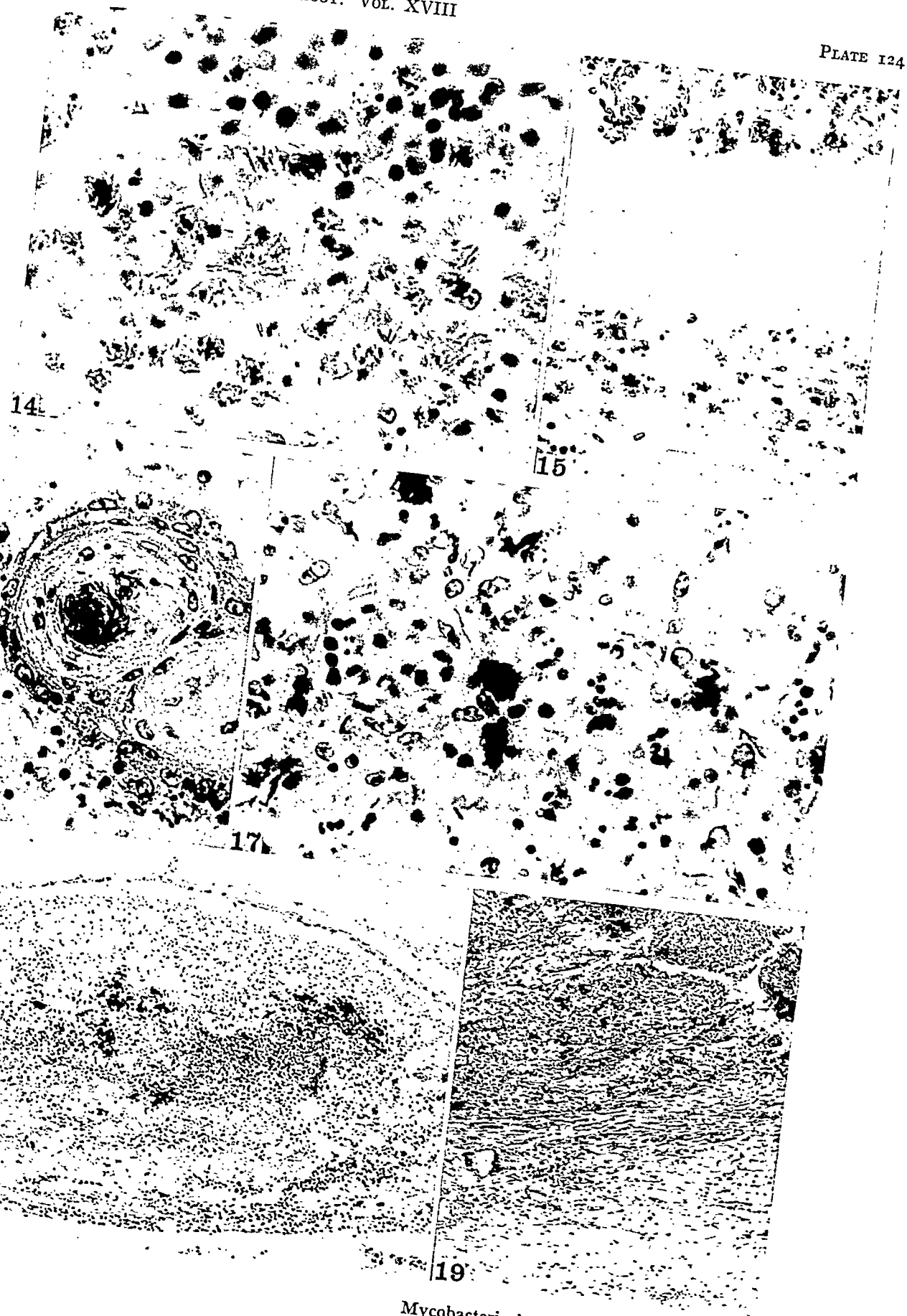
FIG. 9. Tubercle formation caused by *Mycobacterium tuberculosis bovis*. There is an increase in the number of capillaries. Acid-fast stain. × 145.

FIG. 10. Tubercle formation by *Mycobacterium tuberculosis bovis*. Giant cells, capillaries and cellular infiltrate are shown. Acid-fast stain. × 350.

FIG. 11. Tubercle caused by *Mycobacterium tuberculosis bovis*. Note "pearl" and numerous bacilli. Acid-fast stain. × 955.

FIG. 12. Mesodermal nodule showing numerous virulent bacilli of *Mycobacterium tuberculosis avium*. Acid-fast stain. × 95.

IG. 13. Intracellular virulent bacilli of *Mycobacterium tuberculosis avium* in mesodermal nodule. Acid-fast stain. × 470.



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PLATE 124

FIG. 14. Phagocytized bacilli of a less virulent strain of *Mycobacterium tuberculosis avium*. Acid-fast stain. × 900.

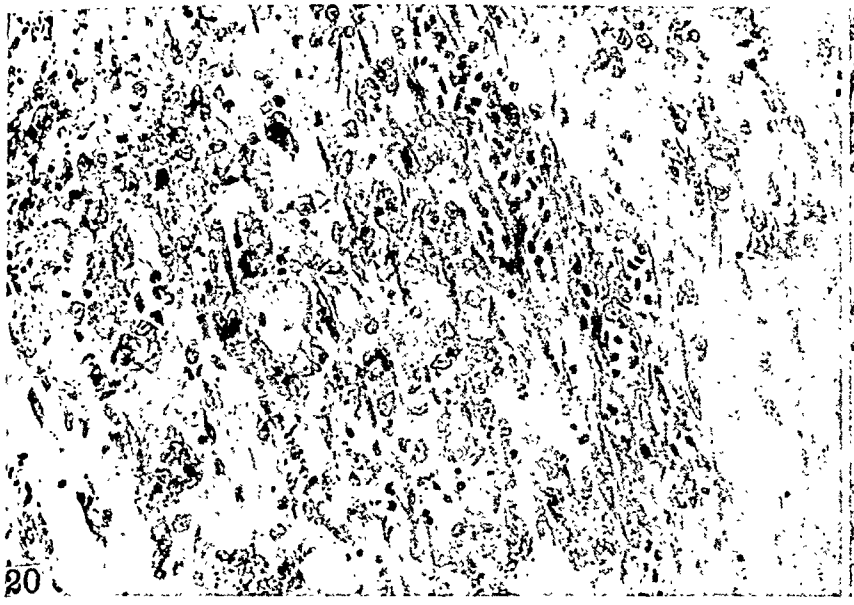
FIG. 15. Section through membrane showing separation of mesoderm from ectoderm. There is a foreign body response of macrophages at base of ectoderm and phagocytized bacilli in mesoderm. *Mycobacterium tuberculosis avium*. Acid-fast stain. × 420.

FIG. 16. Bacilli of *Mycobacterium marinum* in core of ectodermal "pearl." Acid-fast stain. × 525.

FIG. 17. Mesodermal response to *Mycobacterium marinum*, showing clumps and isolated bacilli and reaction around capillary. Acid-fast stain. × 700.

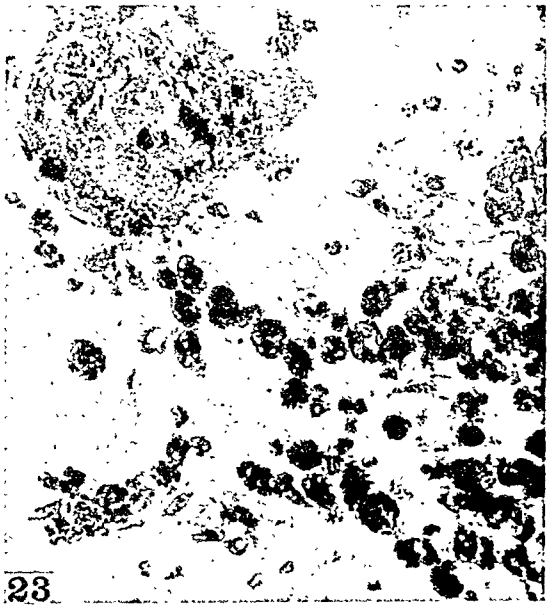
FIG. 18. Rapid invasion of mesoderm by *Mycobacterium marinum* on fourth serial passage. Acid-fast stain. × 130.

FIG. 19. Membranal response to inoculation with *Mycobacterium thamnopheos*. Giant cells and fibroblasts may be seen. Acid-fast stain. × 80.

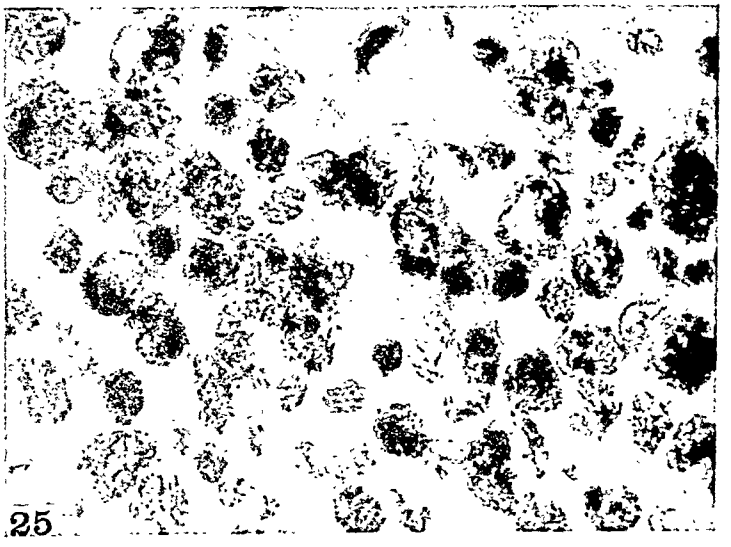

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PLATE 125

FIG. 20. Section through mesoderm, showing giant cells and increased number of capillaries. *Mycobacterium thamnopheos*. Acid-fast stain. × 360.

FIG. 21. Fused giant cells (tubercle) produced by *Mycobacterium thamnopheos*. Acid-fast stain. × 365.

FIG. 22. Section through membrane inoculated with *Mycobacterium leprae muris*. There is ectodermal reaction and invasion of mesoderm by bacilli. Acid-fast stain. × 120.

FIG. 23. Intracellular bacilli of *Mycobacterium leprae muris* in lepra or "foam" cells and in ectodermal extension. Acid-fast stain. × 445.

FIG. 24. Lepra or "foam" cells. Hematoxylin and eosin stain. × 500.

FIG. 25. Intracellular lepra bacilli. Acid-fast stain. × 840.

SENILE INVOLUTION OF THE THYROID GLAND *

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INTRODUCTION

It is the purpose of the present work to describe the changes occurring in the thyroid gland as a result of the aging process. Studies on the histology of the thyroid in senility are not wholly lacking in the literature, but both the observations and the interpretations drawn from them frequently are at variance and at present give anything but a clear-cut picture. This may be due, at least partly, to the fact that the human gland most frequently has been the object of study; and it is impossible with such material to eliminate factors of malnutrition, race and environment, and pathological condition. The present study confines itself to one experimental animal, the mouse, and chiefly to members of one pedigreed strain of that animal.

LITERATURE

In 1933 Dogliotti and Nizzi-Nuti [1] studied the thyroid glands of 53 individuals, 9 of whom were 70 years of age or older. On the histological side, they found reduction in the size of follicles with a corresponding decrease in amount of colloid, and an increase in the volume of the epithelial cells, with an increase in their content of granules. From these findings they were led to believe that there is in old age actually an increased degree of activity of the thyroid gland.

Again, in 1935, they [2] reported a study of the thyroids of 50 aged subjects, upholding their earlier conclusions. Speaking of the findings of a very early investigator on the subject (Clerc,[3] 1912), they said: "Alterations of regressive or degenerative type, such as described by Clerc in advanced age, have been observed by us at any period of life, when the cause of death was a serious infectious or exhausting disease." (Clerc had listed as characteristic senile changes: degeneration of epithelial cells, with fat and pigment appearing in them, a "concentration" or solidification of the colloid, and a more or less noticeable increase in connective tissue.)

Hinton [4] studied thyroids of 80 individuals varying from newborn to 89 years of age, dying from accidental causes. In his group there were only 2 over 60 years of age and the histological picture was quite variable. He stated: "So far we have been unable to draw conclusions as to what is normal thyroid tissue for the different ages." Rice,[5] on

* Received for publication, November 18, 1941.

Materials and Methods

Twenty animals were used in this investigation, the majority of which were pedigreed black mice of the stock C57, purchased from the Jackson Memorial Laboratory at Bar Harbor, Maine. These animals can be divided conveniently into three groups: the young, the middle-aged and the senile.

In the young group there were 5 animals: 4 white mice, 2 females and 2 males, each 50 days of age, from a local laboratory, and 1 C57 black female mouse, 41 days of age, from the Jackson Memorial Laboratory.

The middle-aged group included 6 animals: males of 362 and 381 days, and females of 312 and (3) 381 days each.

The senile group included 9 animals: males of 706 and 719 days, and females of 586, 595, (2) 596, 693, 696 and 699 days.

The animals were kept in our laboratory for several days after arrival, receiving adequate food and water supply during this time. They were then killed by rapid etherization and the thyroid glands were removed, together with the larynx and the upper portion of the trachea, in order to disturb the structure of the gland as little as possible. The fixative employed was a 4 per cent solution of formaldehyde. Lowe,[10] after many trials, found this to be the most satisfactory fixative for the colloid, and we have seen practically no shrinkage attributable to it.

The tissues were then washed, dehydrated, infiltrated and embedded in paraffin. Frontal sections of the entire block of tissue were then cut at 8 to 10 μ, and the sections mounted and stained with hematoxylin and eosin. Large numbers of sections and various portions of the glands of each animal were observed. Both qualitative and quantitative studies were pursued. Numerous sections were observed and 500 or more follicles from each gland were given special study.

Observations

Sections of the thyroid glands from members of each of the three groups designated as young, middle-aged and senile displayed characteristics even under the low-power ($\times$ 100) which enabled one to distinguish them without difficulty.

In the thyroids of the young animals the follicles presented a very uniform appearance, both as to size and structure. Most of the follicles ranged from 30 to 50 μ in internal diameter; the maximum diameter being 85 μ. All of them were lined with cuboidal epithelial cells with spheroidal, vesicular nuclei. These cells possessed a compact, rather homogeneous, and readily stainable cytoplasm, with no vacuolation. The interstitial material was fairly abundant and consisted chiefly of immature adipose tissue. It tended to separate groups of follicles rather

the other hand, believed that definite criteria for normality at different ages can be established. Garau [6] found evidence of increased activity in old age in the thiner colloid in numerous follicles with high epithelial cells, as such thin colloid, according to his concept, is colloid recently formed. This increased activity in places he apparently considers as compensatory to the degeneration of follicles elsewhere, which are "smothered" or submerged by the proliferating connective tissue.

Nolan [7] reported on a very extensive series of autopsies (725 cases). He found in this series that as age increased the number of "normal" glands decreased, and he said: "Fibrosis is relatively more common with increase in age." His tables, however, subdivided his cases on the basis of pathological condition without giving the age of the individuals. Hertzler [8] found in old age an increase in the basophilic property of the colloid, some abortive attempts of follicles to reproduce themselves and changes involving the connective tissue. In general he regarded the changes seen as frankly regressive. Ghigi and Reggiani,[9] in a study on constitutional types, age and the thyroid, found in older persons a tendency to a stabilized condition of the thyroid, numerous larger follicles with "colloide statica" and a lining of squamous epithelium. They found also an increase in interfollicular masses of epithelial cells.

In one of the few studies on age changes in the thyroid in animals other than man, Lowe,[10] working with sheep, came to a similar conclusion. The condition of the secretion in which it appears solid and probably more or less "static" she designated as the "colloid condition." She found this to be the ordinary condition in her older animals, together with, in some, a thickening of the interfollicular connective tissue. Her "older animals," however, varied from "1 year 9 months, to 4 years and over."

An investigation which approached the problem of senile changes in the thyroid from a different aspect is that of Vogeler.[11] He used as a criterion for the activity of the thyroid glands the minimum amount of dried, powdered gland which would induce transformation of tadpoles. By this method he found a slight increase in activity of the thyroid in old men, as compared to those of middle age, and in old women he found no apparent decrease in its activity.

One recent paper (Smith and Starkey [12]) has dealt with age changes in the thyroid gland of the normal mouse, using large numbers of animals, ranging from birth to 250 days of age. Although their oldest animals were chronologically younger than our "middle-aged" animals, they found large areas of cystic degeneration in the glands of that group.

angular, rather than presenting the round or oval forms which were the only shapes found in the younger age-groups. In addition to the irregularly shaped follicles, there were numerous groups of epithelial cells surrounded on all sides, as made out by study of serial sections, by dense fibrous connective tissue. These we believed to be the remains of follicles. Certainly, in a given volume of gland there were far fewer follicles in the senile than in the middle-aged mice. While there usually was no sign of a lumen in these interacinar epithelial cell groups, the arrangement of the cells was sometimes very suggestive of "smothered" follicles.

In addition to the great variety in shape and in colloid content of the follicles in old age, there were such differences in size that minute follicles, 20 to 30 μ in internal diameter, were seen beside enormously distended follicles, reaching a diameter of 400 to 500 μ. In such distended follicles the epithelium consisted of greatly flattened cells, and where the thin walls of these large follicles came together, rupture had occurred in many instances, so that bilocular or trilocular follicles were distinguished (Fig. 6). The epithelial cells of the senile follicles showed profound changes as compared to those of younger animals. The higher cuboidal cells showed nuclei still vesicular but of less intense staining qualities than formerly and irregular or "shrunken" in shape. The cytoplasm was very pale and highly vacuolated, with the more stainable portion forming a coarse meshwork of strands or gathered near the base of the cell (Fig. 4). The nuclei of the squamous epithelial cells were pyknotic.

All of the interfollicular substance was fibrous connective tissue with nuclei of connective tissue cells lying with their axes parallel to the direction of the fibers.

With high magnification, it was possible to see in many of those follicles which under low-power seemed entirely empty, a tenuous coagulum of material occupying part or all of the follicle and probably representing, it seemed to us, the remains of a thin fluid present in the follicle during life. Such an appearance was seen also about the periphery of the solid, fissured masses in the center of many of the follicles.

Quantitative data on the thyroids were obtained by a study of 500 or more sections of follicles in each animal. Table I gives a comparison between some characteristics of the follicles in the 20 animals studied. This comparison, it should be noted, is actually between sections of follicles rather than whole follicles, although the term "follicle" is used for convenience in the table. However, the classification of 500 such sections should give a fairly accurate idea of the general histological picture of the gland. In classifying it was customary to use every other section on the slide.

than individual follicles from one another. The follicles, almost without exception, contained large amounts of colloid of a rather lightly acidophilic nature, many of them being completely filled and many having a mass of homogeneous colloid in the center, with a space between the colloid and the cells. The colloid, at least in this fixed material, was seen to be of an almost homogeneous but very finely vacuolated structure, the entire body of the colloid being of this type.

The thyroids of the animals in the middle-aged group were different in many respects from those of the young animals. In the middle-aged animals the follicles occupied almost the entire bulk of the gland, with only small amounts of interfollicular material showing between them. The follicles were on an average much larger than those of the young animals, although the range in size was considerable. The maximum internal diameter of these follicles was 175 μ. The colloid here was considerably more eosinophilic than in the younger animals. It filled the follicles and seemed even to distend them, most of the larger follicles being lined with a very low cuboidal or definitely squamous type of epithelium. The colloid in these follicles was homogeneous. The cytoplasm of the epithelial cells was well stained, the nuclei of the definitely cuboidal cells were vesicular and those of the squamous cells pyknotic. The interstitial material, only very small amounts of which were present, was similar to that in the young mice.

The appearance of the thyroid glands of all of the senile animals was very different from that found in either the young or the middle-aged animals. The two most conspicuous features were, first, the great increase in definitely fibrous connective tissue and, second, the loss of a large proportion or of all of the stainable colloid from very large numbers of the follicles. These changes were definitely more marked, in our series of animals, in the females, although the male animals showed them in varying degree. The follicles presented a remarkably varied aspect, even within the same field. There were many apparently empty follicles; others showed a few shreds of colloid or small clumps of granular material; still others presented considerable amounts of colloid in the form of concentric rings, the "layers" of which differed sharply in staining capacity. In this latter group the central mass, conforming more or less to the shape of the whole follicle, was at times of a reddish purple tinge, evidently possessing some basophilic quality as well as its eosinophilic property. The outer layers stained much less deeply and appeared less solid, frequently showing a minute vacuolation (Fig. 3). In some places the central mass was split, reminding one of fissures in hardened jelly, while this was never true of the more lightly stained outer layers. The empty follicles, and also those with only small amounts of colloid remaining, were frequently distorted, elongated or

sparse content of colloid in the follicles in many of their senile specimens. Garau [6] thought that evidence of increased activity might be noted in some epithelial hypertrophy and in the presence in numerous follicles of a very thin colloid, supposedly of "recent" formation.

We believe that the small amount of stainable colloid seen in the old animals means rather that the gland has gradually been depleted of this substance. The very thin, fluid colloid seen then to fill or partially fill many follicles would represent, in all likelihood, colloid in process of absorption through the epithelial cells to the blood vessels rather than recently formed secretion. The presence of the very solid-appearing and frequently split colloid in some follicles would tend to support this view of a more static condition of the gland, with secretion now overbalanced by reabsorption, but even that proceeding less rapidly, perhaps, than in the younger animals.

In regard to the interacinar collections of epithelial cells, we agree with the opinion of Garau [6] that these represent degenerate follicles, robbed of their stored secretion, no longer capable of secretion and forced into mere clumps of cells by the encroaching connective tissue.

The enormous distension of some of the follicles in the senile animals, with the extreme flattening of their epithelial cells, and in some instances the rupture of one or more follicles into one another, is an interesting phenomenon. It may be similar to the phenomenon which Smith and Starkey [12] described as "cystic degeneration" in their older animals. While their "old" animals were less than half the age of ours, they were using a different type of mice—albino mice of their "Hygienic Strain"; and it seems possible that the life-span may vary in different strains of mice. Their observations on the histological character of the thyroid in young and middle-aged animals agree well with ours in regard to the amounts of colloid and of interfollicular material and to the size of the follicles and nature of the epithelial cells lining them.

While senile changes of the same general type occur in both sexes, these changes were far more marked among the females in the animals which we have studied. Further work is needed to show whether this apparent sex difference is a constant one.

It has not been the purpose of the present study to attempt to solve the problem of the mechanism of thyroid secretion, but some of the findings here reported seem so pertinent to that subject that we shall discuss them briefly.

A question frequently recurring in the literature is that of the reality in life and the significance of the vacuoles in the colloid. Some recent papers by authors using very modern methods of investigation tend either to deny the existence or to minimize the importance of such

Discussion

We feel that the present study shows a definitely regressive process, a true histological involution, of the thyroid gland in senility. This process appears similar to involution as seen in other glands, for instance, the mammary gland. There is a great increase in the amount of connective tissue; such an increase that the follicles frequently appear like islands in this proliferating fibrous material.

Table I

Classification of 500 or More Sections of Follicles in Each of 20 Mice According to the Amount and Type of Colloid Present

Animal, age in days and sex	Total no. of follicles classified*	Full†		Partially full‡		Partially full§		Empty	
		No.	Percentage of total no. of follicles	No.	Percentage of total no. of follicles	No.	Percentage of total no. of follicles	No.	Percentage of total no. of follicles
41 ♀	543	528	97.2	0	0	15	2.8	0	0
50 ♀	647	269	41.6	0	0	374	57.8	4	0.6
50 ♀	500	91	18.2	0	0	404	88.8	5	1.0
50 ♂	529	369	69.8	1	0.2	157	29.7	2	0.4
50 ♂	519	251	48.4	1	0.2	260	50.1	7	1.3
312 ♀	551	545	99.0	2	0.3	4	0.7	0	0
362 ♂	556	544	97.7	2	0.4	8	1.4	2	1.4
381 ♂	517	492	95.2	0	0	24	4.6	1	0.2
381 ♀	585	507	86.8	2	0.3	72	12.3	4	0.7
381 ♀	526	305	58.0	2	0.4	190	36.2	29	5.5
381 ♀	529	310	58.6	17	3.2	198	37.4	4	0.8
719 ♂	503	330	65.6	14	2.8	152	30.2	7	1.4
706 ♂	506	190	37.5	12	2.4	295	58.3	9	1.8
586 ♀	520	18	3.5	6	1.2	434	83.5	62	11.9
595 ♀	559	8	1.1	6	1.1	376	63.5	169	30.2
596 ♀	522	18	3.4	10	1.9	366	70.1	138	26.4
596 ♀	505	11	2.2	15	3.0	335	66.3	144	28.5
693 ♀	527	36	5.8	10	1.9	315	59.8	166	31.5
696 ♀	585	19	3.2	2	0.3	235	40.2	329	56.2
699 ♀	537	85	16.4	6	1.2	235	45.5	191	36.9

* All follicles are here classified as they appear under low power (× 100).

† Those follicles in which the stainable colloid completely fills the lumen.

‡ Those follicles in which the colloid completely fills the lumen but in which it is in the form of two or more rings, of different staining capacity (Fig. 3).

§ Those follicles in which an apparently empty space, large or small, surrounds the colloid which may itself vary from fairly abundant to very sparse in amount.

Dogliotti and Nizzi-Nuti [1,2] found no noteworthy increase in such connective tissue in thyroids of senile human beings. Others, however, have suggested it. Clerc [3] had stated that an increase in connective tissue occurs in old age. Rice [5] found an increase in interacinar connective tissue and also of interacinar groups of epithelial cells. Hertzler [8] found "changes involving the connective tissues"; and Garau [6] found varying degrees of increase in this element. Nolan,[7] on the other hand, concluded that it was not possible from his studies to say what is normal thyroid tissue for different ages.

Dogliotti and Nizzi-Nuti [1,2] believed that a compensatory hyperfunction of the thyroid occurs in old age, basing this opinion partly on what they thought to be epithelial proliferation and, more largely, on the

intracellular droplets demonstrated by De Robertis represent colloid in process of being secreted.

The present study on the nature of the colloid at different stages in the life cycle shows a striking lack of homogeneity in this substance in old age, a period in which the amount is greatly diminished, without new secretion to balance the loss. It is at this period that we would expect, then, to see visible evidence of the changes in the colloid prior to reabsorption. Our observations and figures demonstrate that wherever there is lack of homogeneity in the colloid, the thinner, minutely vacuolated parts are nearer to the periphery, while the more solid mass, which we might almost conceive as "melting down" into the fluid portion, lies in the center of the follicle. We readily admit that the chromophobic vacuoles seen by many earlier authors, after various types of fixation, may be artefacts, but the great lack of homogeneity of the colloid in the thyroids of senile mice could not be interpreted as artefactual.

Conclusions

1. A study of the thyroid gland in young, middle-aged and senile mice shows marked qualitative and quantitative differences. These differences are greater between middle-aged and senile animals.

2. The thyroids of young animals show relatively small follicles, cuboidal epithelial cells and an almost homogeneous colloid present in large, though varying amounts. There is a fairly large amount of interfollicular material, consisting chiefly of immature adipose tissue.

3. In middle-aged animals the follicles are larger on the average, with low cuboidal or squamous epithelium, and a homogeneous colloid which fills the lumen. There is very little interfollicular connective tissue.

4. In senility there is an enormous increase in the fibrous connective tissue between the follicles. There is an atrophy of some follicles, a hyperdistension of others. The colloid presents an extremely varied picture. Many follicles lack stainable colloid entirely. Others contain only traces, sometimes in clumped granular form. Others show definite stratification, with solid, fissured colloid in the center and thinner, finely vacuolated colloid at the periphery and next to the epithelial cells.

5. The thyroid gland of the mouse undergoes a definite histological involution in old age.

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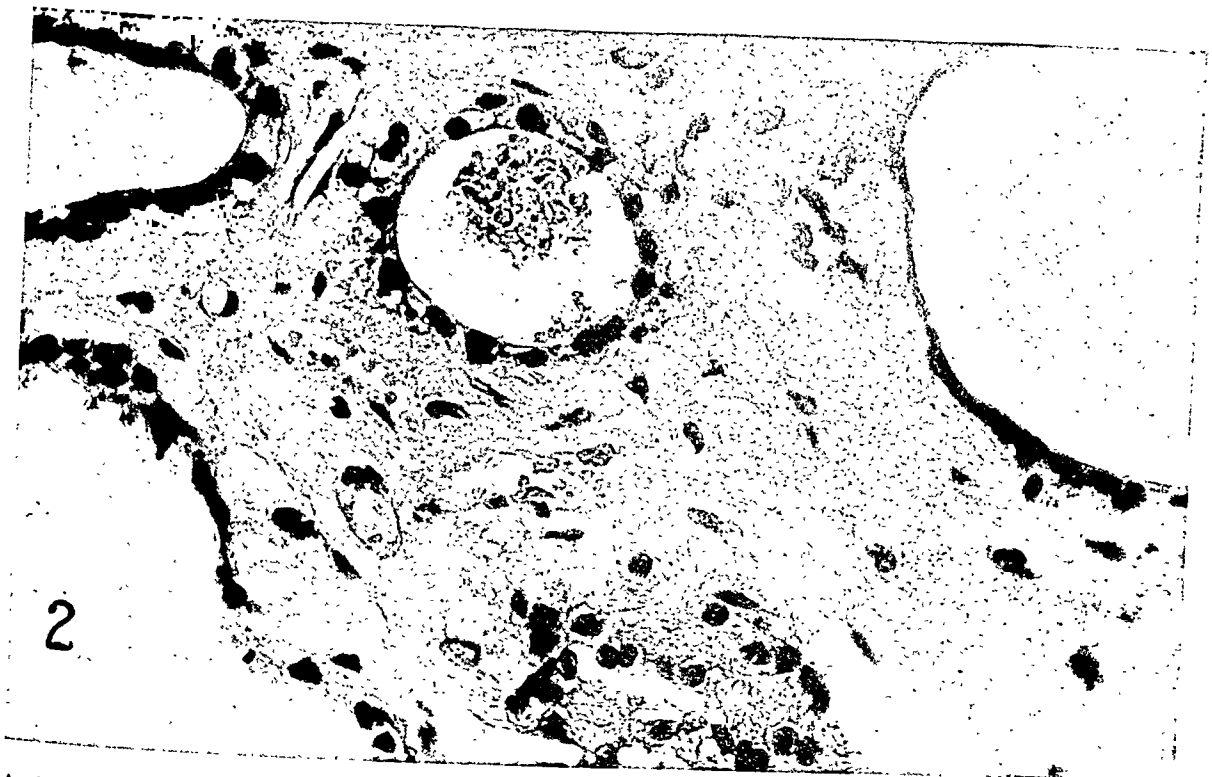
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vacuoles. Gersh and Caspersson [13] analyzed the colloid of thyroid follicles of guinea pigs for total protein and iodine content, using the absorption of ultraviolet light by this substance to determine its chemical nature in areas as small as 0.5 square μ. They stated: "No evidence whatever could be uncovered indicating significant unhomogeneity of the colloid." This refers to the colloid within any given follicle. From follicle to follicle, the colloid differed considerably in chemical constitution.

Williams,[14] in studies on living follicles, has observed a definite cycle of changes in the size of the lumen and the thickness of the wall. He, too, was inclined to minimize the functional significance of apparent lack of homogeneity of the colloid. He said: "That the colloid may escape from holes in the wall or by definite channels in or between the cells is not indicated by these studies." It would seem that the elimination of these alternatives would leave some type of digestion or liquefaction and absorption of the colloid, through the epithelial cells, as digested foods are carried through the cells of the intestinal tract, as a likely method. It is rather interesting in this regard that the active principle of the thyroid gland is not destroyed by digestive enzymes, as are the hormones of many other glands. Embryologically, of course, the thyroid gland is a derivative of the upper part of the digestive tract, along the walls of which many glands with digestive secretions are developed. In this regard, a new concept developed by Leach [15] which suggests that the endostyle of ammocoetes has at least two functions: first, the formation of a secretion which mixes with the food, and second, the absorption of certain elements dissolved in the water circulated by its ciliated cells, is of considerable interest, as suggesting an original dual rôle for the nonciliated cells of the thyroid in secretion and absorption. This idea of secretion with later digestion and reabsorption is not entirely new. Thus Gersh and Caspersson [13] said: "The colloid in the lumen may be digested by the action of enzymes secreted there by the cells."

De Robertis,[16] using young and "normal adult rats," also stressed the homogeneity of the intrafollicular colloid, as seen after preparation of the tissue by the freezing-drying method. He believed that chromophobic vacuoles, when seen, are usually artefacts of fixation. Colloid release, according to this author, is brought about by transcellular transport of the intrafollicular colloid and also, when the release is very active, by inclusion of colloid by the thyroid cells. The presence of such demonstrable colloid droplets in the cells, however, could hardly be said completely to contradict the idea of alteration of the colloid before its reabsorption by these cells. Certainly many of the





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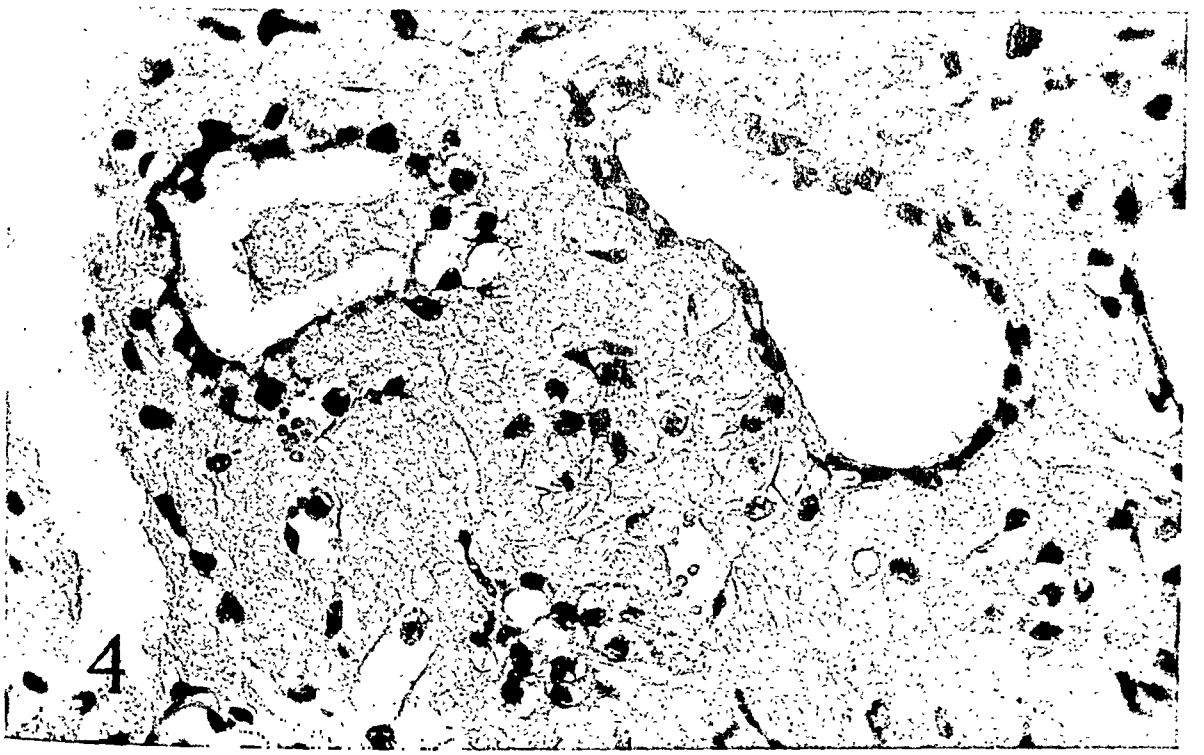
DESCRIPTION OF PLATES

PLATE 126

FIG. 1. Thyroid gland of a 41-day-old female mouse. The follicles are filled with a finely vacuolated colloid. The epithelial cells are fairly high cuboidal, and the interstitial material, present in considerable amount, but very loosely meshed, consists of immature adipose tissue. × 470.

FIG. 2. Thyroid gland of a senile mouse, a 596-day-old female. There is a large amount of dense fibrous connective tissue. The follicles are, for the most part, only partially filled with stainable colloid or are entirely empty. The colloid is variable in appearance. The mass of epithelial cells in the lower central portion of the field is probably a degenerate follicle. × 470.



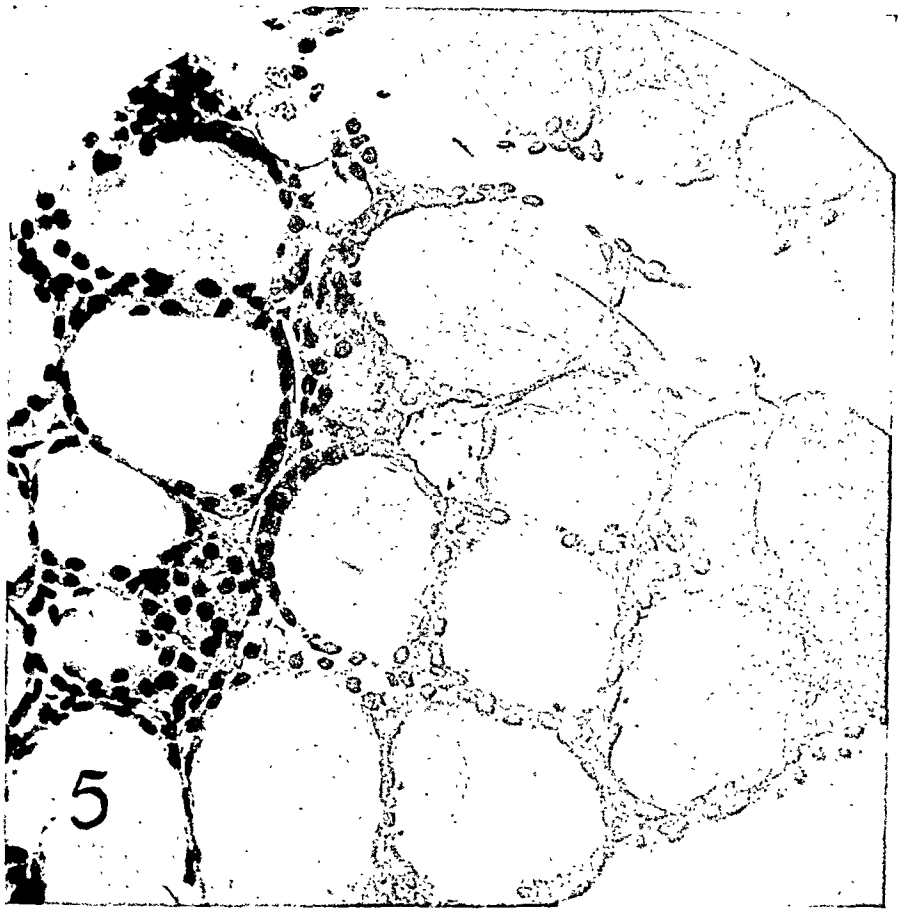


Andrew and Andrew Senile Involution of the Thyroid Gland

PLATE 127

FIG. 3. Thyroid gland of a senile mouse, a 693-day-old female. The follicles in the field show the very solid-appearing central colloid and the thinner, vacuolated colloid which is apparently a step toward the absorption of the material. × 470.

FIG. 4. Thyroid gland of a senile mouse, a 595-day-old female. The larger of the two follicles shown has an elongated, irregular shape and is empty of stainable colloid. The smaller follicle, on the left, shows a small amount of thin, vacuolated colloid still present. There is a large amount of interfollicular connective tissue and the epithelial cells show degenerative changes. × 470.





Andrew and Andrew Senile Involution of the Thyroid Gland

PLATE 128

FIG. 5. Typical field from the thyroid gland of a mouse of middle age, a 312-day-old female. The follicles are filled with homogeneous colloid, with low cuboidal epithelial cells lining the lumen. × 235.

FIG. 6. A group of follicles from a senile mouse, a 596-day-old female. Here rupture of the greatly stretched epithelium has occurred in several places and a group of communicating follicles has been formed. × 235.

PERIPHERAL NERVES IN CHRONIC ATROPHIC ARTHRITIS*

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Occasionally in diseases of unknown etiology, organic pathologic changes are found which do not manifest themselves clinically. Recently the muscles and valves of the heart in chronic atrophic arthritis have been examined and noteworthy changes have been described.[1] At the suggestion of one of us an investigation of the central and peripheral nervous system in cases of chronic atrophic arthritis was undertaken. In the 2½ years of our investigation, previously undescribed pathologic changes have been found. This communication reports these findings.

At the beginning of this investigation of the involvement of the nervous system in chronic atrophic arthritis, only brains and spinal cords were examined. No specific or characteristic lesions were found. Later, in addition to the central nervous system, peripheral nerves and the sympathetic chain were studied. Fourteen cases diagnosed as chronic atrophic arthritis came to autopsy. A study of the clinical features and the pathologic findings of 3 of the cases revealed that the clinical diagnosis was questionable. Thus 11 cases remained in which the clinical and the pathologic diagnoses were established beyond doubt. Roentgenograms confirmed the diagnosis in each case and showed narrowing of the joint spaces, sharply edged margins of the joints, destruction of subchondral bone, atrophy of epiphyseal bone and occasional swellings of periarticular soft parts. The central nervous system was examined in each of these 11 cases. In 5, the peripheral nerves also were examined.

The nerves were fixed in a 4 per cent solution of formaldehyde. The following stains were used in histologic examination: hematoxylin and eosin, van Gieson, Giemsa (Lillie), cresyl-violet, Bodian's stain for axis cylinders, Tibor Pap's stain for reticulin,† all on paraffin-embedded material; Spielmeyer's myelin sheath stain, and fat stain (scarlet red) on frozen sections. Frozen sections were usually made; the remaining tissue was brought back into formaldehyde and then cut into smaller blocks which were embedded in paraffin and stained. Forty to 70 paraffin blocks of peripheral nerves from each case were examined.

* Aided by a grant from The Children's Fund of Michigan.
Received for publication, December 26, 1941.

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toxylin or Mallory's aniline blue as well as van Gieson's stain revealed collagenous strands between the homogeneous masses, but likewise no typical fibrin reactions. Reticulin stain revealed a number of fibrils between the masses. Giant cells or multinucleated isolated cells were not seen. The separation into three zones was not sharp. There was usually a more or less indistinct merging, particularly between the intermediate and peripheral zones. The central homogeneous acellular zone was sharply demarcated, however, from the intermediate zone.

Deviations from the regular three-zonal arrangement were often observed. For example, there sometimes was no acellular central zone; only the intermediate epithelioid cell zone and the peripheral lymphocytic ring. In other instances the inner zone contained a central blood vessel which in its outer walls, particularly in the adventitial parts, was filled with the usual amorphous and acellular material. The intermediate zone was then smaller than is usually seen or was represented by a few epithelioid cells. A cross section through the nerve showed the typical perineural location, a thickened arteriolar wall, and acellular masses in a small part of the vessel wall located in the adventitia and also more peripherally (Fig. 4). There were particles of nuclear débris only in the part of the vascular wall near the acellular zone. A slight deviation from the usual appearance showed the epithelioid intermediate zone not in the center but in a more peripheral location. A loose network, but not a typical lattice of connective tissue, was present at the periphery of the acellular central masses. The nerve fibers themselves, as shown in Spielmeyer's stain for myelin sheaths, in Bodian's stain for axis cylinders and in sections stained for neutral fat (scarlet red), were normal throughout. In this case 65 different single nodules were examined from 30 paraffin blocks. Sections of 16 of these blocks showed nodules; sections of 14 blocks were negative. This gave an average of 4 nodules in 1 positive section.

Case 2

Z. F. was a white female, 63 years old, having atrophic arthritis of 16 years' duration, with involvement of hands, knees and toes; right upper lobe pneumonia, and hypertension.

Pathologic Examination

The following pathologic diagnoses were made: chronic atrophic arthritis, lobar pneumonia, marked generalized arteriosclerosis, severe coronary sclerosis, nephrosclerosis and multiple small hemorrhages in both thalami and occipital lobes due to hyalinosis of the walls of the arterioles.

Peripheral Nerves. Sixty-five different paraffin blocks were cut from four nerves (sciatic, femoral, tibial, axillary). Inflammatory nodules

In some instances threads were attached to each end of the nerve. The threads were inserted into small glass tubes and the nerves then submerged in a jar containing a 4 per cent solution of formaldehyde for fixation in a perfectly straight direction.

A clinical and pathologic summary of each of the five cases in which the peripheral nerves were examined follows:

Reports of Cases

Case 1

A. S. was a white female, 55 years of age, with arthritis of 18 years' duration. This patient showed involvement of most of her joints with stiffness and finally ankylosis; parchmentlike, scaly, and in places shiny and atrophic skin, and some rigidity of the spine in all segments. The patient was subjected to manipulative procedure under anesthesia and in 1 hour and 20 minutes developed deep breathing and cyanosis. Death occurred 2 hours after operation.

Pathologic Examination

The following pathologic diagnoses were made: chronic atrophic arthritis, chronic rheumatic heart disease with mitral valvulitis and pericardial adhesions, fat emboli of lungs and kidneys, chronic passive hyperemia of viscera, recent small hemorrhages in the floor of the fourth ventricle of the brain and fat emboli without hemorrhages in the brain and the spinal cord.

Peripheral Nerves. Femoral and tibial nerves from both sides were examined. In cross sections as well as in longitudinal sections stained with hematoxylin and eosin, small nodules of dark color were seen with the naked eye. They were 0.1 to 0.3 cm. in longitudinal diameter and shorter in the transverse diameter, so that a rather regular oval shape resulted. There were usually two to three such nodules in one slide. However, slides of adjacent sections were frequently devoid of these nodules. The nodules were located almost exclusively in the perineurium, never in the endoneurium and rarely in the epineurium. Figure 1 is a characteristic picture of this location between two sheets of the perineurium, representing a cross section through the peripheral nerve. A histologic analysis of these nodules showed that they consisted of three different zones: a central acellular zone of homogeneous coagulated material occasionally containing nuclear débris; an intermediate zone consisting of large, irregularly shaped, hypochromatic nuclei with more or less definite nucleoli, and a peripheral outer zone containing numerous lymphocytes and plasma cells (Figs. 2 and 3). A sharp demarcation of the lymphocytic peripheral zone from the surrounding normal peripheral tissue was always present. The homogeneous central masses did not take the typical blue-black color of fibrin in Weigert's differential stain. Other stains such as phosphotungstic acid hema-

cated and they contained more collagenous bundles in the intermediate zone than were seen in other cases. There was a diffuse thickening of the perineural coverings of nerve bundles. One inflammatory nodule in epineural fat tissue was seen. A peculiar finding in this case was a perineuritic nodule encasing the total circumference of a small isolated nerve bundle. Here only lymphocytic infiltration was seen. The lymphocytes did not enter the endoneurium; they were not seen between the nerve fibers of this bundle. The intermediate zone was seen in a number of nodules. There was a zone of characteristic epithelioid cells, but there were numerous collagenous fiber bundles in this zone. Occasionally the intermediate epithelioid zone was more eccentrically located than usual. No nerve fiber changes were found in myelin sheath, axis cylinder, or fat stains.

Case 5

E. B., a white male, 49 years of age, had atrophic arthritis of 7 years' duration, with involvement of hands, shoulders, knees, ankles and hip; chronic iritis and secondary glaucoma, and chronic pyelitis.

Pathologic Examination

The following pathologic diagnoses were made: atrophic arthritis; septicemia due to hemolytic streptococcus with acute bacterial mitral and aortic valvulitis, and myocarditis; amyloidosis of liver, spleen, kidneys, adrenals, lymph nodes; chronic bilateral pyelonephritis; congenital atrophy of superior pole of left kidney with compensatory hypertrophy of right kidney. No focal lesion of the brain was found.

Peripheral Nerves. Forty-seven blocks were cut from 3 nerves (right and left femorals, right axillary). Twenty-two single nodules were found in 18 different blocks. Sections from 14 blocks showed only 1 nodule in each section. Here again, location, shape and demarcation were similar to those in previous cases. The nodules were located exclusively in the perineurium. They were oval or ellipsoid and sharply circumscribed. An acellular central zone was not present, but the intermediate and peripheral lymphocytic zones were seen. In this case epithelioid cells and lymphocytes were more intermingled; a distinct separation of the intermediate zone from the peripheral lymphocytic zone was in most instances not so clearly defined as in other cases.

Kahn and Kline tests of blood were negative in all cases.

Morphology of the Lesions

The findings in the peripheral nerves can be summarized as follows: The principal characteristic finding was a *nodular inflammatory* lesion. This lesion consisted of accumulations of lymphocytes and plasma cells

were seen in only five blocks. Again, the characteristics were the location in the perineurium and the sharp demarcation from the surrounding tissue. These nodules, however, showed only massed lymphocytes and plasma cells. The central acellular zone and the intermediate epithelioid zone were not seen. Occasionally a few epithelioid cells were observed as a rudimentary feature of an intermediate zone. No changes in the nerve fibers were seen with fat, myelin sheath, or axis cylinder stains.

Case 3

M. R., a white female, 73 years of age, had atrophic arthritis of 4 years' duration, with involvement of the elbows and of the metacarpal-phalangeal joints; tenderness of the left shoulder, left wrist, knees and ankles; cellulitis of the right elbow with septicemia due to hemolytic streptococci.

Pathologic Examination

The following pathologic diagnoses were made: atrophic arthritis, septicemia, septic spleen and liver, coronary sclerosis and occlusion with myocardial infarct.

Peripheral Nerves. Forty-two different paraffin blocks were cut from two nerves (femoral, tibial). Only two nodules were found of the pure lymphocytic type described in case no. 2. Here also the perineural location and the nodular character were noted.

Case 4

M. D. was a white female, 61 years old, with the duration of her atrophic arthritis not known. She showed the following: typical fusiform swelling of fingers of right hand; arthritis of both wrists and elbows; bilateral ulnar deviation; Heberden's nodes on distal phalangeal joints; hypertensive heart disease; enlargement of heart to left; blowing apical and aortic systolic murmurs; blood pressure, 170/138 mm. of Hg.

Pathologic Examination

The following pathologic diagnoses were made: atrophic arthritis, hypertrophic arthritis, coronary thrombosis of anterior descending branch, recent myocardial infarct, aortic and coronary sclerosis, thickened mitral valve, arteriolar nephrosclerosis, healed tuberculosis of lung, chronic salpingitis and hyperemia of the leptomeninges. No focal lesion was found in the brain.

Peripheral Nerves. Thirty-eight different blocks were cut from two nerves (femoral, axillary). Twelve single nodules were found; three in the same slide from one block, the others in single slides from nine different blocks. In these nodules there were two distinct zones, the intermediate and the peripheral lymphocytic zone. The central acellular zone was absent. The nodules were oval or spherical. They were, with the exception of one which was epineural, situated between perineural folds, as is seen in Figure 5. The nodules were sharply demar-

alone or combined with the inner polymorphous cell layer, with cells of the epithelioid cell type. The size of the three zones varied: the middle layer of epithelioid cells and the innermost homogeneous acellular central zone were usually smaller than the outer lymphocytic ring.

Of special interest are the so-called epithelioid cells. These were mesodermal cells, the nuclei of which resembled the nuclei of the perineural mesodermal elements. The nuclei were elongated, poor in chromatin and almost always irregularly shaped. As seen in cresyl-violet, methylene blue or Giemsa (Lillie) stains, epithelioid cells formed a symplasm. Clear-cut cell outlines were not demonstrable, but there was a faintly stained cytoplasm (Fig. 6). The origin of these epithelioid cells is obscure. Their derivation from the endothelial cells of the blood vessel walls or from other vascular mural cells is not probable for the reason that there is occasionally, in the innermost homogeneous zone, a blood vessel with its walls completely unchanged, and for the reason that the epithelioid cell layer is often not connected with peripheral zones of the vascular wall. There is a complete separation of the vascular cells from the epithelioid cells by the homogeneous acellular masses.

From serial sections it was seen that some of the apparently pure lymphocytic nodules had an epithelioid center. Homogeneous masses, however, were not regularly found even by serial sections. For this reason it was assumed that they represent a more transitory phase of the nodule. Since serial sections could not be made of every nodule, the statement that there was an epithelioid cell center in *each* nodule may be questioned. However, in eight out of ten serial sets, the epithelioid inner cell layer was found even when the sections made first showed only lymphocytic nodules.

The sharply circumscribed appearance of the nodules should be emphasized. The outer lymphocytic ring was sharply demarcated from the surrounding perineural tissue, which was entirely normal. Hence the use of the term "nodule" is justified.

In each of our five cases of chronic atrophic arthritis in which peripheral nerves could be examined, similar changes were found. Cases nos. 1, 4 and 5 showed well developed identical lesions; in cases nos. 2 and 3 the lesions were more rare and were not fully developed. There seems to be a relationship between the age of the patient and the severity of the lesion, for the younger patients showed more severe lesions than the older ones. In cases nos. 1, 4 and 5 the patients were 55, 61, and 49 years of age respectively; in cases nos. 2 and 3 the patients were 63 and 73 years old. Since the findings in the peripheral nerves were not foreseen and these lesions were not expected, no par-

in the perineural nerve sheath or, less frequently, in the adjacent epineural tissues. The nodule cannot be seen grossly in the unstained specimen, but with hematoxylin and eosin and with other methods of staining, it was seen with the naked eye as a small round or oval nodule. Occasionally the nodule covered the entire surface of a small nerve bundle, uniformly infiltrating the perineurium and its outer regions without penetrating the nerve bundle itself and its endoneurium. The perineuritic nodules were often found in groups of three or four in the same section. They were scattered through a portion from 2 to 2.5 cm. of the longitudinal course of a peripheral nerve. Very often the adjacent blocks of the same nerve were completely free from these nodular lesions. There was no specific distribution of the nodules except their location in the perineurium.

The size of a single nodule varied. Serial sections through such a nodule in paraffin-embedded material showed an extension of a nodule through 20 to 25 sections of 7 μ each; that is, 140 to 175 μ (0.14 to 0.175 mm.). The maximum diameter observed was 0.2 mm. Assuming that the embedding process leads to a general shrinkage of the tissues by use of dehydrating agents and xylol, the original size of the nodule may be somewhat larger.

A more detailed histologic analysis of the nodule showed a complicated pattern consisting of two or three different layers. The first was a lymphocytic outer zone with rather numerous plasma cells in the most peripheral part; the second was a middle zone of polyhedral cells having large and irregular nuclei poor in chromatin, cells comparable to the epithelioid cells in gummatous or other granulomatous tissues; the third and innermost zone consisted of a pale and *acellular* center with homogeneously staining deposits in which occasionally small dark granules of nuclear detritus were embedded. Capillaries and other small vascular channels were found in all parts of the nodule, even in the innermost homogeneous part where well preserved vascular walls were seen. The endothelial cells of the vascular walls were normal or slightly swollen, and the adventitial spaces of the vessels were occasionally filled with the same homogeneous material which had spread without clear-cut limitation into the immediately adjacent structures. Various stains to identify the homogeneous deposits showed that this material did not give a definite fibrin reaction. It stained deep purple with hematoxylin and eosin and greenish with cresyl-violet. Reticular fibers ran through it. In some places true collagenous fibers were seen in outer portions of the deposits. In other nodules in the same section the homogeneous center was lacking, and only one or both of the distinct outer zones were found; *i.e.*, the peripheral lymphocytic zone

cases of chronic atrophic arthritis; 1 of parkinsonian syndrome, probably of postencephalitic origin; 2 of dermatomyositis; 1 of Buerger's disease and 1 of bronchogenic carcinoma. A detailed morphologic study of the findings in the peripheral nerves furnished data for a better differential diagnosis between the nerve changes in chronic atrophic arthritis and in other diseases.

Control Case 4

The patient was a white male, 61 years old, who was first admitted to the hospital on April 14, 1940 complaining of chronic arthritis. The clinical history included a positive Kahn reaction of the blood in 1927; the patient received some antisyphilitic treatment.

Peripheral Nerves. Both femoral nerves were available and 25 blocks were embedded. Only two nodules were found, one in fat tissue between two nerve bundles and another in the perineurium of a nerve bundle. This second nodule was seen grossly in stained preparations. It was more spindle-shaped than the nodules usually are. Serial sections of this nodule showed a three-zonal arrangement; a very small central acellular zone containing homogeneous material, a larger intermediate zone with numerous epithelioid cells and a peripheral zone with numerous, densely packed lymphocytes and plasma cells. The epithelioid cells showed dumbbell-shaped and small elongated nuclei. Somewhat different from perineuritic nodules found in other cases of chronic atrophic arthritis, this nodule was located beneath the outer parts of the perineural sheath of the nerve bundle and directed itself toward the endoneurium (Fig. 7). However, it was clearly seen that the adjacent nerve fibers were separated from the nodule by a thin layer of perineural connective tissue fibers. No invasion of the endoneurium was seen, even in serial sections. An unusual finding was that of one giant cell located in the peripheral part of the nodule; no other giant cells were seen (Fig. 8). There were no degenerative changes in the nerve fibers.

Control Case 46

Illness began in April, 1940 with migratory joint pain in knee, hip and elbow; no swelling or redness. The following findings were noted: generalized muscle aching, some numbness and tingling of lower extremities; generalized vitiligo and chloasma; in the extremities, some interosseous atrophy with a suggestion of clubbing of the fingers; some evidence of atrophy in the lower extremities; foot drop, especially on the right; inability to flex the head; weakness of all muscles in arms, legs, and back. The patient developed respiratory paralysis, which was relieved by respirator ("iron lung"). Tuberculosis with large areas of caseation necrosis was found in an axillary lymph node.

ticular attention was given to a correlation between the degree of involvement of the various *joints* of the four extremities and the severity of lesions in the *peripheral* nerves. The nerves collected were those which could be removed during autopsy without too extensive additional cutaneous incisions (axillary, sciatic, tibial, femoral and sympathetic chain). Only nerve trunks were removed. The more peripheral portions close to the joints were not examined. Therefore, nothing can be said about the intensity of the peripheral nerve changes near the joints, or about the relationship between the severity of the regional joint lesions and the lesions in the peripheral nerves. The small number of nerves examined (five cases) does not permit us to say anything about the relationship between the *duration* of chronic atrophic arthritis and the involvement of peripheral nerves. The most severe involvement of the peripheral nerves was seen in cases nos 1, 4 and 5. In case no. 4 no reliable history of the duration of the disease was available; in cases nos. 1 and 5 the duration was 18 and 7 years respectively. In cases nos. 2 and 3 the duration was 16 and 4 years.

Specificity of the Lesions

In order to establish the specificity of the observed morphologic lesions, 86 control cases were examined.

Our control material was studied without knowledge of the clinical findings or of the pathologic diagnosis. The autopsies were done by members of the respective departments of pathology, and the nerves were sent to the neuropathologic laboratory with the autopsy numbers on the containers but without other data. Normal nerves were found in 79 cases. The diseases represented by these cases can be summarized as follows: malignant tumors, 21 cases; vascular diseases (particularly arteriosclerosis, arteriolar sclerosis and hypertension), 23 cases; rheumatic heart disease, 4 cases; neurosyphilis and other organic diseases of the central nervous system (multiple sclerosis, postencephalitic parkinsonism), 5 cases; Buerger's disease, 2 cases; hypertrophic osteoarthritis, 3 cases; miscellaneous diseases such as lobar pneumonia, miliary tuberculosis, subacute bacterial endocarditis, pyelonephritis, chronic nephritis, hydronephrosis, pyosalpinx with consecutive peritonitis, liver cirrhosis (2 cases), peptic or duodenal ulcers (3 cases), volvulus of sigmoid, thyrotoxicosis, polycythemia vera, diabetes mellitus, Gaucher's disease, pemphigus foliaceus and skull fracture, 21 cases. It should be emphasized that in *4 cases* of *chronic rheumatic heart disease* as well as in *3 cases* of *hypertrophic osteoarthritis*, the peripheral nerves were found to be normal.

Lesions were seen in the peripheral nerves of 7 cases. These were 2

Pathologic Examination

The following pathologic diagnoses were made: coronary sclerosis with myocardial fibrosis; cardiac hypertrophy with dilatation; old healed myocardial infarct; bilateral degeneration of the melanin-containing nerve cells in the substantia nigra.

Peripheral Nerves. Both femoral nerves were obtained and 45 blocks were cut, in nearly all of which identical lesions were found. There was a diffusely spread endoneural inflammatory infiltration with lymphocytes (Figs. 9 and 10), an infiltration which followed the course of the endoneural blood vessels. No nodules were found; no zonal pattern was seen. The perineurium was involved only rarely. When it was involved, the same elongated lymphocytic infiltration along the course of the blood vessels was seen. Occasionally degenerative changes in the nerve fibers were found, showing myelin sheath destruction, ballooning of axis cylinders and production of fatty granular material.

Control Case 78

A lower extremity was amputated for thromboangiitis obliterans. The left tibial nerve (from the surgical specimen) was examined. An outstanding feature of this case was the vascular change. Enormous thickening of all layers of the vascular walls (Fig. 11), complete occlusion and occasional subendothelial deposition of homogeneous material were seen. Marked fibrous hyperplasia of the perineurium was found together with massive epineural and perineural accumulations of lymphocytes and numerous plasma cells, usually perivascularly arranged (Fig. 12). Single plasma cells were scattered through the epineurium and the perineurium. No zonal patterns of the lymphocytic accumulations and no nodules were found. The nerve fibers showed various degenerations identical with those reported in 1938 by Barker.[3]

Control Case 81

Both femoral nerves of this case of bronchogenic carcinoma with metastases were examined. They showed infiltration of the epineural fatty tissues with numerous islands of tumor cells but no infiltration of the perineurium or the endoneurium. However, there were definite degenerative changes in the nerve fiber bundles themselves, with myelin sheath degeneration and production of catabolic granules of fat for certain distances along isolated nerve fibers.

Control Case 83

In this patient with dermatomyositis, in muscles as well as in the epineurium and perineurium changes were found identical with those seen in control case no. 46.

The seven control cases with pathologic findings in the peripheral nerves showed various lesions. One case of chronic atrophic arthritis revealed a picture almost identical with that found in our five cases of chronic atrophic arthritis. Another control case of chronic atrophic arthritis showed arteriolar changes in the perineurium of peripheral

Pathologic Examination

The following pathologic diagnoses were made: dermatomyositis; chronic thyroiditis and extensive destruction of thyroid gland; caseous tuberculosis of axillary and submaxillary lymph nodes and spleen, and miliary tuberculosis of liver and spleen. There was degeneration of muscle fibers with fibrous replacement. Many fibers were swollen and eosinophilic. Lymphocytes and plasma cells were seen in the interstitial muscular tissues and were present in large groups in fat adjacent to the muscle.

Peripheral Nerves. No lesions with either the three-zonal or the two-zonal arrangement, or with the sharp nodular demarcation were seen in the perineurium or epineurium. There were occasional degenerative lesions in the peripheral nerves. The pathologic findings were similar to those described by Kinney and Maher.[2] Single plasma cells were seen scattered between the inflammatory accumulations in the perimysium and toward the perineurium. Areas of the epineurium and the perineurium adjacent to inflammatory foci in the muscles showed accumulations of inflammatory cells.

Control Case 52

The patient presented the following: chronic atrophic arthritis; marked flexion at the hip joints as well as at the knee joints, with considerable atrophic deformity of toes; marked ulnar deviation of hands with marked deformity of all fingers, characteristic of atrophic arthritis; nearly complete destruction of cartilage of both knee joints; partial bony ankylosis with contraction; fixation of patella of both knee joints.

Pathologic Examination

The following pathologic diagnoses were made: hypertensive heart disease; cardiac hypertrophy; marked coronary sclerosis; aortic atherosclerosis; small arteriosclerotic softening in putamen with no other focal lesions in the brain.

Peripheral Nerves. There were changes in the walls of the arterioles in the perineurium and hyalinized thickening and occasional complete occlusion of perineural and epineural arterioles. Singly scattered plasma cells were seen around the blood vessels (perivascularly arranged) but none in the vessel walls themselves. There were no degenerative changes in the nerve fibers.

Control Case 53

The patient presented a history of parkinsonian syndrome starting 3 years previously, with a slight pill-rolling tremor of the hands which gradually increased. There was also trembling of head and jaw, "lead-pipe" rigidity of all extremities and a slight arthritis of the right hand and left shoulder.

have rarely been examined. There is only one report. Koeppen [4] (1932) reported changes in the sciatic nerves in three out of seven cases of rheumatic fever. In two cases (one of subacute rheumatic fever with pancarditis and one of rheumatismus nodosus, both with typical Aschoff bodies in the heart muscle) he found circumscribed perivascular lymphocytic infiltrations located in the endoneurium of the sciatic nerve or more rarely in the perineurium with invasion into nerve bundles. In a third case of rheumatic fever the endoneural lymphocytic infiltrations were scarce. In a second group of healed cases of rheumatic fever, among them five cases with healed rheumatic carditis, no lesions in the sciatic nerves were seen. In two cases of arthritis deformans (Table I, case 8; Table II, case 9), one with rheumatismus nodosus and the other with healed endocarditis, the sciatic nerves were found normal. The investigation of Koeppen seems to indicate that there are inflammatory endoneural nodules in the sciatic nerves during the acute or subacute stage of rheumatic fever, nodules which disappear, however, after the acute phase has subsided. There seems to be a discrepancy between the findings of Koeppen and our findings in so far as his examination of the sciatic nerves in two cases of chronic atrophic (postrheumatic) arthritis was entirely negative. (5) There is a striking resemblance between the histologic lesions in the peripheral nerves in chronic atrophic arthritis and the subcutaneous nodules in the same disease. There has been ample discussion about the similarity or dissimilarity between the subcutaneous nodules in rheumatic fever and those in chronic atrophic arthritis (Hawthorne,[5] Findlay,[6] Dawson,[7] Collins,[8] Keil,[9] McEwen,[10] and recently, Bennett, Zeller and Bauer [11]). We are not concerned with this problem, but rather with a comparison of the lesions of the peripheral nerves with the subcutaneous nodules in chronic atrophic arthritis.

The subcutaneous nodules are larger and more irregular in shape than the perineuritic nodules. Notwithstanding this difference, they are alike in that they both show three well-defined zones: the central zone of necrosis, the intermediate zone of proliferating mesenchymal cells and the peripheral ringlike zone of inflammation with lymphocytes and plasma cells. In perineuritic nodules the central area of necrosis is not very well developed; there is more often a small "fibrinoid" area containing nuclear débris rather than a large necrotic homogeneous mass. Conglomeration into larger areas as seen in subcutaneous nodules has not been found in perineuritic nodules. There is an occasional loosening of the intercellular substance in the central zones of perineuritic nodules but not the lattice formation seen in subcutaneous nodules. Palisading of epithelioid cells in the intermedi-

nerves with small collections of inflammatory cells located perivascularly but not in the adventitia.

In the case with Parkinson's disease an interesting lesion was found in the peripheral nerves. It showed no nodular pattern and was entirely different from the changes seen in peripheral nerves of cases of chronic atrophic arthritis. The lesion found was designated as diffuse lymphocytic endoneuritis.

In the two cases of dermatomyositis the epineurium and the perineurium were not involved, with the exception of regions in which the adjacent muscles, the perimysium and the interstitial tissues surrounding muscle bundles were diseased. The inflammatory change in epineurium and perineurium was produced by an invasion of the inflammatory process from the neighboring muscular tissues. Again the nodular pattern was absent. This perineuritic change was considered as secondary to the primary myositis.

In the case of thromboangiitis obliterans, vascular changes were seen which were entirely different from the findings in other diseases. The inflammatory lesions were diffusely scattered and particularly marked in the vascular walls and in the perivascular portions. The definite nodular pattern was entirely lacking. The endoneurium was not involved, but definite degenerations of nerve fibers were seen.

In the last case metastatic tumor cells were seen in the epineurium and isolated degenerations of nerve fibers, but no inflammatory cells.

The question arises as to whether the changes in the peripheral nerves in chronic atrophic arthritis are *specific*. There are strong arguments for the conclusion that they are. (1) There is a high incidence of these nerve lesions in cases of chronic atrophic arthritis. They were found in all of 5 cases examined. In 3 of these, they were marked and extensive; in the other 2 cases they were only mild and less characteristic. (2) The lesions are absent in patients suffering from other diseases. In our control material of 86 cases there were 2 cases of atrophic arthritis, 1 with identical findings. Of the remaining 84 cases, 79 showed no pathologic lesions in peripheral nerves. In the 5 cases which showed pathologic changes, the changes were entirely different from those in chronic atrophic arthritis. (3) Distinct location of the nodules in the perineurium or near it in the epineurium, the lack of involvement of the endoneurium, the sharply circumscribed nodular character of the lesions, and the peculiar histologic appearance of the nodules are characteristic and permit differentiating the lesions in chronic atrophic arthritis from nerve lesions in other diseases. (4) These lesions show some similarity to the lesions of the peripheral nerves described in rheumatic fever. Peripheral nerves in rheumatic fever

due to injuries to the peripheral nerves only, were frequently observed,[14] and loss of reflexes and paralysis are common in clinical polyneuritis. In chronic atrophic arthritis, however, the reflexes may be exaggerated. It may be incorrect to emphasize an analogy between chronic atrophic arthritis and polyneuritis, for the muscular degeneration seen in interosseous atrophy in chronic atrophic arthritis (for example in the small muscles of the hands and fingers) is different from the degeneration of muscles seen in polyneuritis. The reaction of degeneration of muscles and fibrillary twitchings are usually not found in chronic atrophic arthritis. Hence muscular lesions in this disease cannot be explained by pathologic changes in the peripheral nerves. Pemberton [12] explained the muscular atrophy in chronic atrophic arthritis by a proliferation of the interstitial connective tissue in and around the muscle bundles and a resultant compression with degeneration of the muscular tissue. Later in the disease, said Pemberton, the muscular tissue becomes largely "displaced and replaced by the newly formed fibrous tissue." Thus, muscular atrophy in chronic atrophic arthritis may be due to injury not originating in the peripheral nervous tissues. The articular and periarticular pathology, the synovial and bony changes and the swelling of the periarticular soft parts seem to be of primary character. So, muscular, articular and periarticular lesions in chronic atrophic arthritis may not be due to peripheral nerve lesions. There remain, however, the pains and paresthesias in chronic atrophic arthritis and the vasomotor, secretory and trophic cutaneous symptoms in this disease which show a definite parallel with those in so-called polyneuritis.

Pemberton [12] has already suggested that the *nervous tissues* in chronic atrophic arthritis *must* be affected in order to give rise to painful symptoms. We now find that there are definite morphologic changes in the peripheral nerves of patients with chronic atrophic arthritis in that the perineurium of the trunks of the peripheral nerves shows a rather regular involvement. We are inclined to consider this change responsible for the pains, paresthesias and trophic clinical symptoms. The examination of the peripheral nerve bundles for degenerative changes of myelin sheaths and axis cylinders was completely negative. Consequently, the sensory and trophic disturbances can be related only to the perineuritic pathologic changes.

There is another strong argument for the correlation of the perineuritic nodules with the pains, paresthesias and trophic disorders. Perineuritic lesions usually cause irritation of the nerve fibers first, whereas endoneural lesions may produce degeneration of nerve fibers. Symptoms of irritation are pain, paresthesias, and vasomotor, secretory and trophic disorders, but symptoms of endoneural lesions with

ate zone is not found in the perineuritic nodules. There is a symplasm of cells, and multinucleated cells and mitotic figures are rare or absent. The peripheral zone in both subcutaneous and perineuritic nodules is characterized by diffuse lymphocytic infiltration.

The conclusion seems to be justified that the reported lesions of circumscribed perineuritis are pathologically specific for chronic atrophic (rheumatoid) arthritis. The pathologic state must be classified as circumscribed perineuritis, or better as *rheumarthritic nodulous perineuritis*. The attribute "nodulous" has been chosen because a multiplicity of nodules is seen with completely circumscribed demarcation of the single nodule. *Nodulosus* (Latin) means rich in nodules, while "nodular" means to have the characteristic appearance of nodules. "Rheumarthritic" is an abbreviation of rheumatoid arthritis.

Clinical Discussion

Pemberton,[12] in his monograph, considered painful symptoms in arthritis due to an involvement of nervous tissues to be either primary or secondary to pathologic processes in adjacent regions. In chronic atrophic arthritis there are pains and paresthesias, vasomotor, secretory and trophic changes in the skin. There is the characteristic clinical picture of diseased bones and joints. As the disease progresses there is muscular atrophy. Pains and paresthesias, such as numbness, often follow the course of a peripheral nerve. Paresthesias other than numbness are the feeling of gloves or of cotton or muslin, pricking, tingling, stabbing, gnawing, or tearing. The pains and paresthesias are increased by touch or pressure on the nerve and by contraction of the muscles. These are symptoms of neurologic disorder. However, neurologic examination of our cases as well as of those reported in the literature showed no disturbances of sensations—touch, pinprick, heat and cold and the deep sensory functions.

There is a striking parallel between the occurrence of pain and paresthesias in chronic atrophic arthritis and in so-called polyneuritis, particularly in regard to their distribution. Polyneuritis usually shows a symmetric bilateral distribution and a more severe clinical involvement of the distal parts of the extremities. Chronic atrophic arthritis commonly shows bilateral symmetry in the form of a marked bilateral involvement especially of distal parts; the hand and fingers, as well as the feet and toes. The involvement of the shoulders in chronic atrophic arthritis amounts to only 9 per cent (Kuhns[13]). When found, it is usually bilateral.

Cutaneous vasomotor, secretory and trophic disorders are not rare in polyneuritis. During the first World War, bone and joint changes,

infectious disease either clinically or at autopsy. One patient (case no. 1) died from fat embolism as a consequence of orthopedic manipulation; the autopsy revealed chronic rheumatic heart disease with mitral valvulitis and pericardial adhesions but no other chronic infectious disease. In case no. 2 lobar pneumonia was the cause of death, and there was coronary sclerosis with hypertension but no chronic infectious disease. Another patient (case no. 3) died from acute septicemia due to hemolytic streptococci. In case no. 4 healed pulmonary tuberculosis and chronic salpingitis were found. In case no. 5 death was due to acute septicemia caused by hemolytic streptococci. Acute bacterial mitral valvulitis and myocarditis, chronic bilateral pyelonephritis and generalized amyloidosis were found. There remain at least the two cases, nos. 1 and 2, in which the findings of perineuritic nodules can be explained only on the basis of chronic atrophic arthritis because no other acute or chronic inflammatory disease was present.

At least three of the eleven cases revealed rheumatic inflammatory processes in the heart (mitral valvulitis) at autopsy, that is, about 27 per cent. A high incidence of rheumatic damage to the heart is to be expected in cases of chronic atrophic arthritis if there is a causative relationship between these diseases. The concomitant finding of rheumatic valvulitis, chronic atrophic arthritis and rheumarthritic nodulous perineuritis in the same body without the presence of any other inflammatory disease, as in case no. 1, points strongly to a common source of the inflammation involving the three different structures; namely, the heart, synovia and peripheral nerves. It is highly improbable that the perineuritic nodules are due to any other agent of inflammatory disease than that of chronic atrophic arthritis.

There are three prominent features of rheumatic pathologic morphology which have to be mentioned: the rheumatic nodules in the heart (Aschoff bodies), the subcutaneous nodules and the "fibrinoid change." Aschoff bodies were not seen in the perineuritic lesions. Bennett, Zeller and Bauer [11] emphasized the finding of a few round or oval lesions of the size of miliary tubercles bearing "a distinct resemblance" to myocardial Aschoff nodules in the subcutaneous nodules of cases of *rheumatic fever*. However, in subcutaneous nodules of cases of *chronic atrophic arthritis*, Aschoff-like nodules were "rarely if ever" seen. None were observed by these investigators in the 67 subcutaneous nodules of atrophic arthritis examined by them. Aschoff,[15] in 1939, considered the rheumatic heart nodule as specific for the granulomata which occur in rheumatism in general. Rheumatic nodules, according to Aschoff, are cell formations specific to rheumatic fever in which they are exclusively seen. Baggenstoss and Rosenberg,[1] investigating cardiac lesions in

loss of function are objective sensory changes and degenerative muscular atrophy. Another point of interest is the migratory and transitory character of the pains and paresthesias which favors again a perineural location more than an endoneural. Thus it seems justifiable to correlate some of the clinical findings in chronic atrophic arthritis with anatomic lesions in the peripheral nerves, *i.e.*, with rheumarthritic nodulous perineuritis. On the other hand, the articular pathologic changes are of primary character, and the involvement of the joints is independent of the perineuritic lesions. Synovial and perineuritic changes should be considered as concomitant changes, possibly due to the same agent.

Pathogenesis

As already mentioned, the pains and paresthesias are considered a consequence of the multiple inflammatory nodules in the perineurium of the peripheral nerves. There is reason enough to assume an irritation of nerve fiber bundles by the space-consuming extension of the nodules, or by other factors not yet known. At any rate, the pathogenesis of pains, paresthesias, and vasomotor, secretory and trophic disorders is now better understood after finding inflammatory perineural nodules in the peripheral nerves.

The pathogenesis of rheumarthritic nodulous perineuritis itself remains obscure. Certainly the perineuritic nodules are not in contact with regions of inflammation in the synovia and other parts of the joints. The changes in the joints and in the peripheral nerves are independent. They may be produced by a common etiologic source, but both are due to the effect of the noxious agent *in situ*.

The perineuritic lesions are also independent of subcutaneous nodules. In our five cases with perineuritic lesions, there were no subcutaneous nodules and the perineuritic nodules were sharply circumscribed and showed no tendency to grow into neighboring tissue structures and to coalesce with each other. These inflammatory foci are of autochthonous origin; they are produced by an infectious or a toxic agent or by a combination of infectious and toxic agents *in situ*.

The perineural inflammatory nodules represent a peculiar type of *active* tissue reaction. It is of a type similar to but not identical with that seen in tuberculosis, syphilis, leprosy and other chronic infectious diseases. The question arises whether the perineural inflammation seen in our cases of chronic atrophic arthritis was produced by the agent of chronic atrophic arthritis or by another and unrelated infectious or toxic agent. The five cases of chronic atrophic arthritis in which the peripheral nerves could be examined showed no conspicuous *chronic*

to the stage of the disease and varies in so far as the specific tissue reaction (subcutis, heart, perineurium) interferes with the potentialities of general defense against the agent.

In principle, the perineuritic nodule in chronic atrophic arthritis shows features identical with those in other rheumatic tissue lesions. There is the "plasma soaking" (Bennett) type of exudation (fibrinoid degeneration or fibrinoid change), the proliferation of mesenchymal (epithelioid) cells and the peripheral infiltration with lymphocytes and plasma cells. There is a division of these histologic characteristics into two or three merging zones.

The pathogenesis of chronic atrophic arthritis is not known. It may be an etiologically uniform disease, or there may be various disease entities producing identical clinical pictures. However, there is a strong possibility that the majority of cases diagnosed as chronic atrophic arthritis are of the same etiology. In the literature two different opinions are advanced. One opinion is that chronic atrophic arthritis is a consequence of previous rheumatic fever, either as a late stage of this disease or as a causally related disease. The other opinion is that chronic atrophic arthritis has nothing to do with rheumatic fever. A definite decision between these two opinions cannot be reached before the agents of both diseases are known.

Recent studies suggest that there is a close relationship between chronic atrophic arthritis and previous rheumatic fever. We refer again to the study of Baggenstoss and Rosenberg [1] concerning cardiac lesions in cases of chronic atrophic arthritis and to the investigation of Bennett, Zeller and Bauer.[11] Baggenstoss and Rosenberg, in examining 25 cases of chronic atrophic arthritis, found cardiac lesions in 20, among them 14 with lesions resembling those produced by rheumatic fever. The rheumatic lesions were *grossly* demonstrable in all but 1 of the 14 cases. Baggenstoss stated that there is a definite relationship between rheumatoid arthritis and rheumatic fever. Bennett, Zeller and Bauer consider the subcutaneous nodules in rheumatic fever and in rheumatoid arthritis as disease-specific and the nodules in both diseases as presenting similar or identical structural and cytologic features.

Rheumarthritic nodulous perineuritis adds one more link to this chain of relationship between rheumatic disease and chronic atrophic arthritis. Since the lesions in the peripheral nerves represent a definite inflammatory change of specific character and since other sources of inflammation or infection can probably be excluded, there remains only one explanation: the perineuritis in chronic atrophic arthritis belongs to the same group of pathologic lesions as are illustrated by the synovial and periarticular inflammation and thickening, the inflammatory and

chronic rheumatoid arthritis, found in 3 cases with subacute cardiac lesions and in 5 out of 7 cases with chronic cardiac lesions, typical myocardial Aschoff bodies. Thus it is definitely established that Aschoff bodies are found in a rather high incidence in the hearts of cases of chronic atrophic arthritis. They are not seen in the subcutaneous nodules or in the perineuritic nodules. Subcutaneous nodules in chronic atrophic and perineuritic nodules in the peripheral nerves in the same disease are in their morphologic appearance different from Aschoff bodies, yet they are disease-specific.

The "fibrinoid" degeneration of Klinge [16] and the "mucinous edema" of Talalajew [17] are two terms for the same pathologic phenomenon. It consists of swelling of collagen bundles of fibrous tissue and an exudation of homogeneous material into intercellular spaces. This is a correct observation beyond any doubt. However, the conclusions in regard to the histochemical character, specificity and significance of this change vary greatly. Klinge considered the fibrinoid change as the early primary change in all rheumatic diseases, followed by focal degeneration, necrosis, mesenchymal proliferation and invasion by inflammatory cells. Collins [8] considered the "fibrinoid" change a prominent and characteristic feature of the rheumatic lesion. However, he called this change the basic type of tissue reaction in states of hypersensitivity. According to Bennett's [18] opinion, the term "fibrinoid degeneration" has received more attention than it probably deserves. He believed that this type of reaction frequently represents a nonspecific type of inflammatory change in connective tissue in which the collagen fibers are separated by exudate. He preferred the term "plasma soaking" to that of fibrinoid degeneration. For rheumatic nodules in the heart, Aschoff [15] himself declared it in no way essential that the formation of the nodules be preceded by fibrinoid degeneration of the intercellular substance.

In our own experience, "fibrinoid degeneration" is not histologically specific for a rheumatic lesion. An identical type of reaction, for example, can be seen in Buerger's thromboangiitis obliterans, particularly in the subendothelial layers of vascular walls. We consider the intercellular and interfibrillar exudation of first liquid and finally genuine coagulating fibrin or fibrinlike (fibrinoid) material as nonspecific for the rheumatic lesion notwithstanding the fact that it is of common occurrence in this disease. No matter which view is accepted in regard to the specificity of the fibrinoid change, there remains the fact that in the subcutaneous nodules of rheumatic fever and of chronic atrophic arthritis, in the Aschoff bodies and in rheumarthritic nodulous perineuritis, granulomata of a peculiar and specific character are found. The histologic appearance of these granulomatous nodules varies in respect

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proliferative nodular changes in the subcutis and the frequent cardiac stigmata of rheumatic type in this disease. This group of lesions indicates a close relationship of chronic atrophic arthritis to rheumatic fever, or better, rheumatic disease, including rheumatic polyarthritis, rheumatic endocarditis, valvulitis, myocarditis and other rheumatic manifestations.

Summary

1. In 3 out of 5 cases of chronic atrophic (rheumatoid) arthritis, characteristic pathologic lesions were found in peripheral nerves. The remaining 2 cases showed only mild lymphocytic inflammation.

2. The location of the pathologic lesions in the perineurium and their sharply nodular and peculiar granulomatous morphology are features of a disease-specific appearance.

3. By examination of peripheral nerves from 86 control cases the specificity of the findings in peripheral nerves of patients with chronic atrophic arthritis has been confirmed. Seven control cases showed pathologic lesions. Two of these were cases of chronic atrophic arthritis, 1 of which showed lesions similar to those found in the 3 cases of chronic atrophic arthritis and the other showed only arteriolar changes in the peripheral nerves. The remaining 5 control cases were 1 of Buerger's disease, 2 of dermatomyositis, 1 of postencephalitic parkinsonism and 1 of carcinomatous infiltration of the epineurium. The lesions of the peripheral nerves in the nonarthritic control cases differed distinctly from the perineuritic changes in chronic atrophic arthritis, even when there was an accompanying inflammatory or infectious disease.

4. The lesion in the peripheral nerves of cases of chronic atrophic arthritis is best designated as "nodulous rheumarthritic perineuritis."

5. Nodulous rheumarthritic perineuritis should be considered a specific active inflammatory process concomitant and parallel with other rheumatic pathologic features in chronic atrophic arthritis, such as rheumatic heart disease, subcutaneous nodules and synovial inflammation and proliferation.

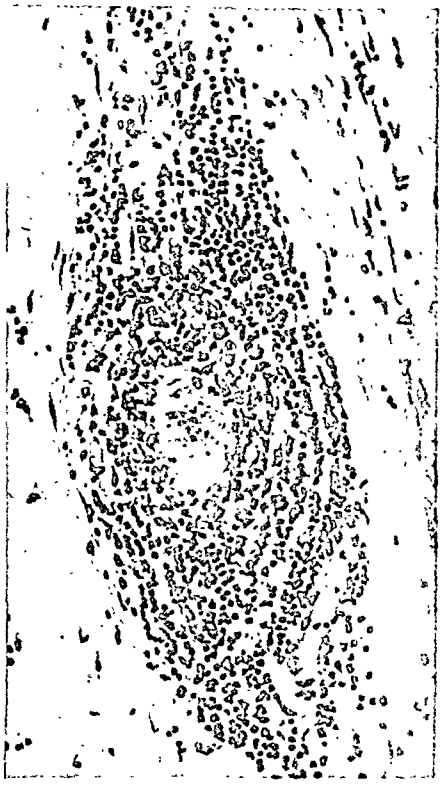
Note: We are indebted to C. Graham Eddy for his photomicrographic work.

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Freund, Steiner, Leichtentritt and Price Peripheral Nerves in Chronic Arthritis

DESCRIPTION OF PLATES

PLATE 129

FIG. 1. Case 1. Cross section through the tibial nerve. Two distinct nodules are marked by arrows. Hematoxylin and eosin stain. × 19.

FIG. 2. Case 1. Femoral nerve. The different zones of one nodule are clearly seen: a central acellular zone with homogeneous material, nuclear débris and a few dark granules; a small intermediate zone consisting of cells with large irregularly shaped hypochromatic nuclei, and an outer peripheral zone of lymphocytes and plasma cells. The nodule is located in the perineurium between two nerve bundles. Hematoxylin and eosin stain. × 150.

FIG. 3. Case 1. Higher magnification of the nodule shown in Figure 2. Hematoxylin and eosin stain. × 420.

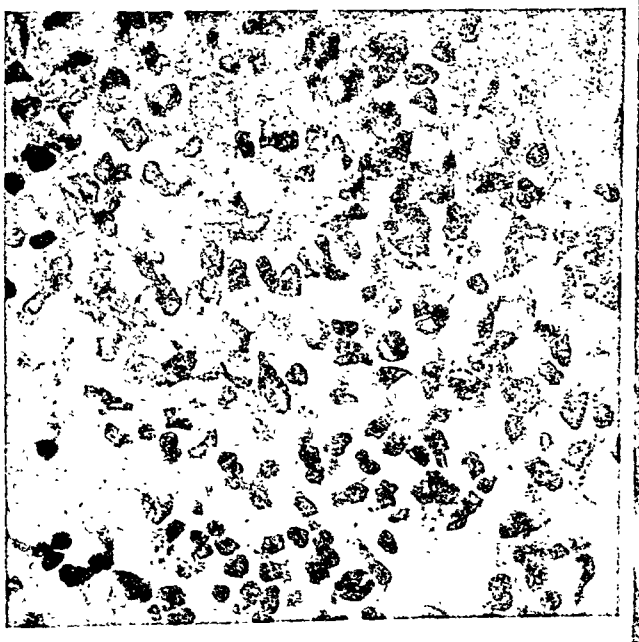
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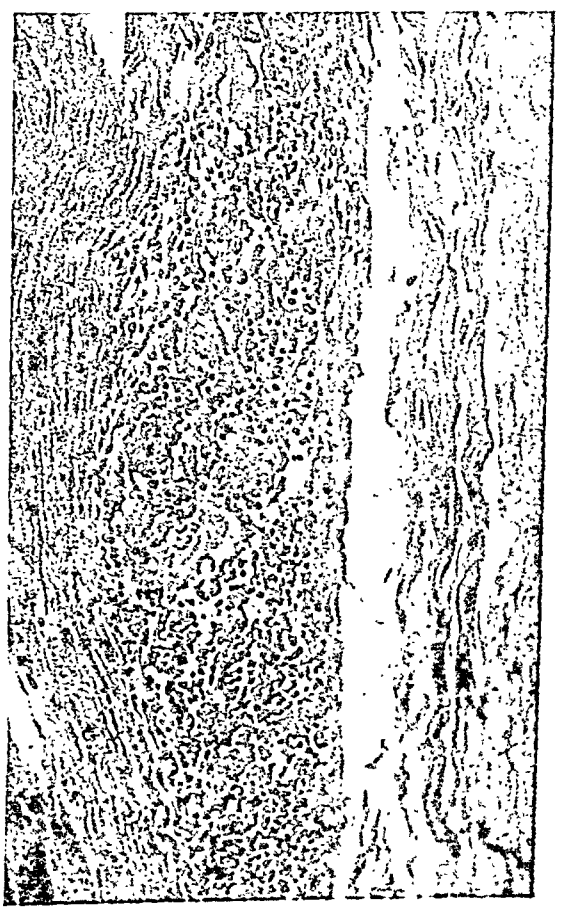
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Peripheral Nerves in Chronic Arthritis

Plate 130

Fig. 4. Case 1. Perineuritic nodule as seen on the lower portion of Figure 1. A thickened arteriolar wall and a small amount of acellular substance in the adventitia are seen. Hematoxylin and eosin stain. × 175.

Fig. 5. Case 4. Femoral nerve. A perineuritic nodule is shown in which the central acellular zone is absent. There are a number of epithelioid cells in the intermediate zone with numerous collagenous fiber bundles giving a somewhat looser structure. Hematoxylin and eosin stain. × 110.

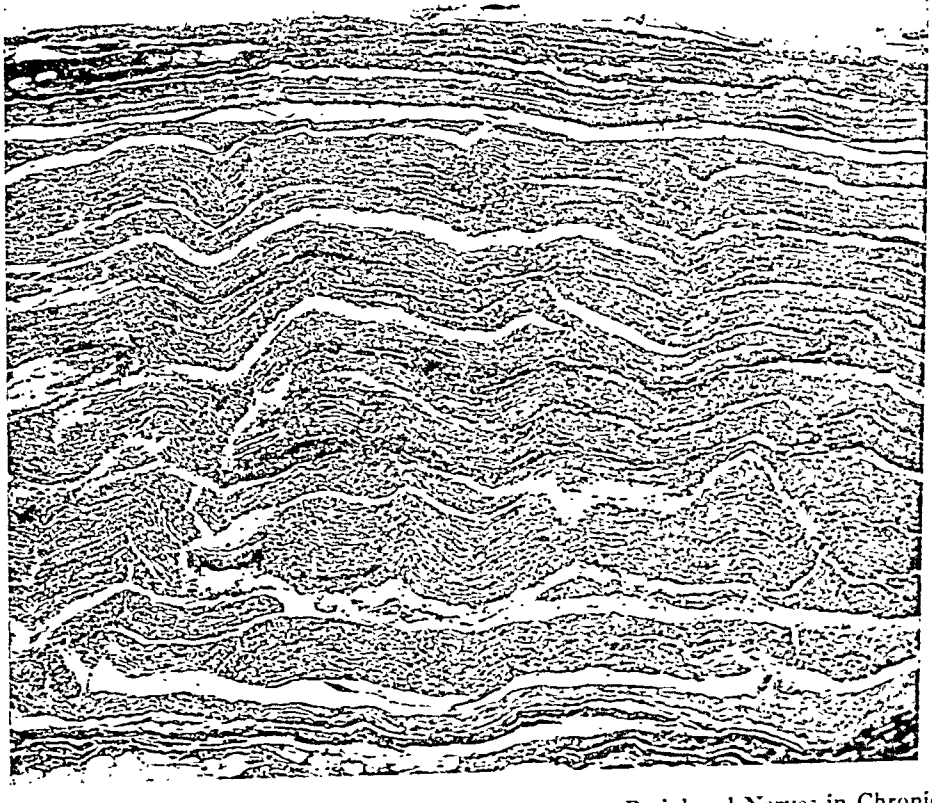
Fig. 6. Case 1. Intermediate zone of a perineuritic nodule. The epithelioid cells show a faintly stained cytoplasm and the demarcation of single epithelioid cells from each other is ill-defined. In the lower right portion of the field, cells of the lymphocytic ring and a few plasma cells are seen. Cresyl-violet stain. × 575.

Fig. 7. Control case 4. Chronic atrophic arthritis. Femoral nerve. Spindle-shaped perineuritic nodule with unusual development toward the endoneurium, but without invasion into the endoneurium. At the top of the picture, one giant cell is seen. Hematoxylin and eosin stain. × 150.

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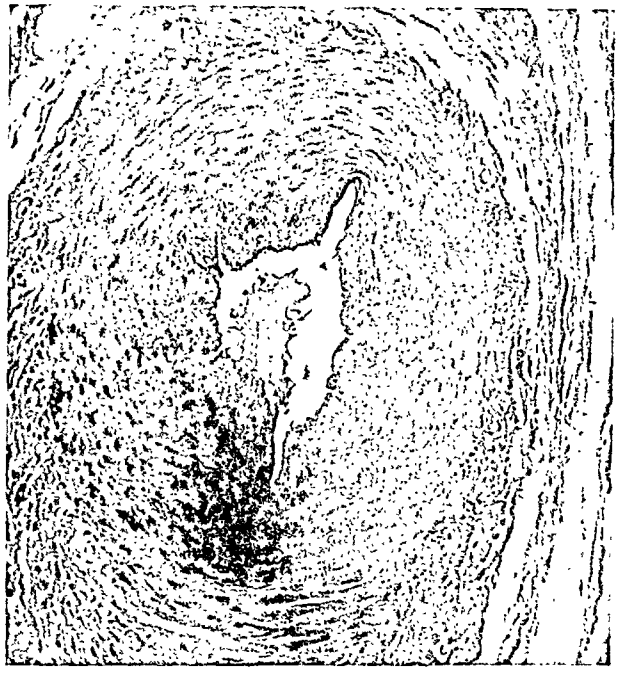
Peripheral Nerves in Chronic Arthritis

PLATE 131

FIG. 8. Higher magnification of the upper portion of Figure 7. × 875.

FIG. 9. Control case 53. Parkinsonian syndrome. Femoral nerve. Diffuse endoneural and moderate perineural lymphocytic infiltration. Hematoxylin and eosin stain. × 50.

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Peripheral Nerves in Chronic Arthritis

PLATE 132

FIG. 10. Higher magnification of one of the areas of lymphocytic infiltration in the femoral nerve used for Figure 9. Hematoxylin and eosin stain. × 175.

FIG. 11. Control case 78. Left tibial nerve. Thromboangiitis obliterans. Marked thickening of the wall of a small artery is seen with perivascular fibrosis. Hematoxylin and eosin stain. × 110.

FIG. 12. Control case 78. Left tibial nerve. Thromboangiitis obliterans. Massive perineural accumulation of lymphocytes and plasma cells without a nodular pattern. Hematoxylin and eosin stain. × 110.

EXPERIMENTAL STUDIES IN CARDIOVASCULAR PATHOLOGY*

V. Effects of Intravenous Injections of Solutions of Gum Arabic, Egg Albumin and Gelatin Upon the Blood and Organs of Dogs and Rabbits

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During the course of experiments with colloidal solutions of several macromolecular substances (polyvinyl alcohol, methyl cellulose) which were tested for their suitability as nonhematogenous blood substitutes, it was observed that they give rise to the development of atheromatous lesions and foam-cellular storage phenomena in various organs, resembling closely those produced by hypercholesterolosis. It was concluded from this evidence and from observations made with vasculotonic agents that degenerative arterial lesions may be elicited by any one of three distinct types of causative mechanisms: (1) vasculotonic agents, causing either a hypertonic ischemic hypoxemia of the vascular wall or a hypotonic stagnant hypoxemia of these tissues; (2) excessive hydrostatic pressure in the vascular lumen resulting from an excessive filling of the vessels with blood (plethora) and producing a hypoxemia of the vascular tissues by an overextension of the vascular wall with collapse of the vasa vasorum and by a compression of the elastic and contractile media against the rigid adventitia; (3) disturbances of the colloidal status of the blood plasma elicited by abnormalities in the quantitative and qualitative physicochemical conditions in this medium and impairing an adequate exchange of gaseous and nutritive elements between the plasma and the vascular tissues by the formation of films or precipitates in the interface (Hueper; Hueper and Landsberg; Hueper, Landsberg and Eskridge; Martin and Hueper; Hueper and Ichniowski).

In further elaboration of these investigations, dogs and rabbits were injected intravenously at frequent intervals with hypertonic solutions of the following three macromolecular colloids: gum arabic, gelatin and ovalbumin. These substances were chosen for the following reasons. Solutions of gum arabic are used extensively in the treatment of nephrotic hypoproteinemia and are known to produce colloidal disturbances of the blood (acute leukopenia, reduction in number of erythrocytes and in amount of hemoglobin, hastened erythrocytic conglomeration and sedimentation, lengthened bleeding and coagulation time, decrease of plasma proteins) and storage phenomena in internal organs including

* Received for publication, January 5, 1942.

distinct reduction in the viscosity of the solution, which also decreased somewhat after moderate autoclaving (Gartside). Viscosity determinations made on gum arabic solutions after autoclaving for 10 to 12 minutes gave the following results: a 3.5 per cent solution had a viscosity of 2.04 at a temperature of 35° C.; a 5 per cent solution, 2.53; a 10 per cent solution, 5.06, and a 20 per cent solution, 18.4 (readings by Hess's viscosimeter).

Eight dogs and two rabbits were used in the experiment. The dogs weighed between 7.12 and 9.17 Kg., and the rabbits weighed approximately 2.5 Kg. at the start of the experiment.

The injection of from 20 to 50 cc. of the 25 per cent solution of gum arabic into the ear veins of rabbits was tolerated without any immediate, untoward symptomatic effects. When, on the other hand, 30 cc. or more of this material were injected into the jugular veins of dogs, these animals invariably reacted with excessive salivation, vomiting and defecation, which appeared 2 to 6 minutes after the introduction of the gum arabic solution. The severity of this reaction was reduced and consisted usually only of excessive salivation, when the amount and the concentration of the gum arabic solution injected was decreased to 10 to 20 cc. of a 10 per cent solution. It was noted that during the course of repeated intravenous injections of gum arabic solution several dogs developed a severe transitory edema of the head, especially in the region of the jaws. The dogs which died exhibited a rapid and marked loss of weight, while those surviving to the end of the experiment maintained their weight.

To study the reactions elicited by the intravenous introduction of gum arabic solution into dogs, 10 cc., 20 cc. and 30 cc. of a 25 per cent solution were injected into the jugular veins of three animals, respectively, and blood studies were made at various intervals thereafter (5 minutes, 15 minutes, 45 minutes, 2 hours, 4 hours, 6 hours, 24 hours, and 2, 3, 7, 8, 10 and 14 days). These three dogs, together with five additional dogs, were then placed upon a regime consisting of daily injections of a gum arabic solution. The pertinent data are summarized in Table I.

The individual doses of gum arabic solution administered to these dogs varied between 20 cc. of a 10 per cent solution to 80 cc. of a 25 per cent solution. The higher doses were given usually toward the end of the experiment and to dogs surviving for more than 75 days.

The two rabbits were injected with a gum arabic solution, the concentration of which was gradually increased from 6 to 25 per cent. One rabbit received 10 such injections within 2 weeks, while the second rabbit received 40 injections within 8 weeks. Both animals died.

the liver, spleen, lymph nodes, kidney and bone marrow (Yuile and Knutti; Heckel, Erickson, Yuile and Knutti; Studdiford; Frimberger; Dick, Warweg and Andersch; Jackson and Frayser; Fukuhara and Hiratsuka; Onozaki and Sanada; Andersch and Gibson; Metcalf and Hawkins; Hall, Gibson and Weed; Falkenstein and Jackson; Christie, Phatak and Olney; Keith, Power and Wakefield; Erickson, Heckel and Knutti), which resemble closely those elicited by polyvinyl alcohol and methyl cellulose.

Clinical as well as experimental observations (Heinlein; Heinlein and Muschallik; Schmitt; Holman; Dick and Leiter; Werley) suggest that abnormalities in the colloidal equilibrium and reactivity of the blood proteins are involved in the production of degenerative and proliferative arterial lesions, and of the colloidoclastic hematic reactions associated with allergic or related responses (hyperglobulinemia, hastened erythrocytic sedimentation and amyloidosis). A study of the relationship between chronic disturbances of the colloidal status and composition of the plasma proteins, induced experimentally by the intravenous injection of foreign proteins (gelatin and ovalbumin), and the development of arteriosclerosis seemed to be indicated, especially as gelatin solutions and modified bovine plasma albumin have been recommended recently for the treatment of shock (Hogan; Taylor and Waters; Cohn; Keys, Taylor and Savage).

Experimental Investigation

I. Gum Arabic

The gum arabic solution used in the experiment was prepared as follows: High-grade gum arabic tears, ground into a fine powder, were dissolved in physiological saline solution by heating on a water bath, giving a moderately opaque, viscous liquid. This material, which contains impurities, was mixed with a large quantity of "Hyflo Supercel"* and filtered while hot through coarse filter paper in a Büchner funnel. The filtrate was almost clear and slightly brown. A 25 per cent solution of gum arabic thus prepared had a pH of about 4. Fifteen to 20 cc. of lime water with a pH of 10 were needed to neutralize 80 to 100 cc. of this solution, reducing thereby its concentration to about 20 to 22 per cent. This neutralized material remained fairly clear after autoclaving for 10 to 12 minutes under 14 to 15 lbs. of pressure, while a moderately heavy precipitate was formed when the time of sterilization was extended to 30 to 40 minutes. This was accompanied by a

* Hyflo Supercel is a preparation of purified diatomaceous earth, obtained from the Dicalite Co., 120 Wall Street, New York, N. Y.

Table II illustrates the changes observed in the dog which received 20 cc. of the 25 per cent gum arabic solution.

Reactions of the Blood after Repeated Injections of Gum Arabic Solution

Following the repeated intravenous introduction of gum arabic solution, changes developed in the blood which followed a certain pattern and sequence and which were subject to some individual variations.

TABLE II

Observations on the Blood after a Single Injection of Gum Arabic Solution

Elapsed time	Hemoglobin	Erythrocytes	Leukocytes	Polymorphonuclear leukocytes	Sedimentation rate (mm)
	18.0	7,730,000	15,400	69	
5 minutes	17.7	7,800,000	7,600	16	24.5
15 minutes	17.9	7,630,000	4,800	14	10.0
45 minutes	16.0	7,260,000	4,300	58	31.0
2 hours	16.0	7,300,000	10,700	84	7.0
4 hours	15.1	6,480,000	8,200	88	6.0
6 hours	14.9	6,870,000	7,200	88	32.0
8 hours	16.4	6,930,000	8,500	83	13.0
24 hours	15.3	6,960,000	9,800	76	30.0
6 days	16.3	7,990,000	14,800	66	1.0
8 days	17.0	8,220,000	14,700	82	2.0
10 days	15.5	6,770,000	10,400	60	1.0

There occurred a gradually increasing and finally severe reduction in the number of erythrocytes, in the volume of packed red blood cells and in the amount of hemoglobin. The sedimentation rate of the erythrocytes became progressively accelerated and ultimately reached a high value. The prothrombin time did not vary appreciably, while the coagulation time was usually moderately to markedly shortened and only occasionally moderately lengthened. The viscosity of the whole blood showed a tendency to decrease because of the increasing anemia, whereas the viscosity of the plasma became simultaneously elevated, reaching pathological value only rarely, however, during the final stage of the treatment, when massive amounts of a highly viscous gum arabic solution were given.

The number of leukocytes underwent a considerable reduction at times and at other times reached values of 300 per cmm. The counting of these cells was severely interfered with during the periods of intensive treatment by the formation of a granular precipitate in the blood treated with acetic acid. Following an initial agranulocytic leukopenic stage, the leukopenia was characterized by a relative increase of the granular leukocytes which sometimes represented up to 97 per cent of the total number of cells identified. The polymorphonuclear leukocytes

Reactions of the Blood after a Single Injection of Gum Arabic Solution

The cellular and physicochemical reactions of the blood of the three dogs after a single injection of 10 to 30 cc. of a 25 per cent solution of gum arabic were identical in character, but varied in intensity with the amount of gum arabic solution administered. They may be summarized as follows:

There developed immediately after the injection a marked but transitory leukopenia and especially granulocytopenia, lasting from 1 to 2

TABLE I
Gum Arabic Solutions Injected into Dogs

Dog no.	Gum arabic solutions injected (total amount)	Gum arabic dry weight	Number of injections	Time of treatment	Death and time after last injection
		(*gm.*)		(*days*)	
729	10% sol., 500 cc. 25% sol., 1,360 cc.	390	47	90	Killed 2 days after last injection
708	10% sol., 840 cc. 25% sol., 750 cc.	274	52	35	Died 5 days after last injection
712	10% sol., 470 cc. 25% sol., 1,460 cc.	415	46	90	Killed 2 days after last injection
701	10% sol., 880 cc. 25% sol., 860 cc.	303	54	78	Killed 25 days after last injection
696	10% sol., 120 cc.	12	4	5	Died on 6th day of experiment
698	10% sol., 240 cc. 25% sol., 90 cc.	43	11	21	Died on 21st day of experiment
605	10% sol., 570 cc. 25% sol., 2,160 cc.	597	31	110	Killed 5 weeks after last injection
681	10% sol., 240 cc. 25% sol., 1,530 cc.	406	47	90	Killed 7 weeks after last injection

hours. This response was followed by a moderate granulocytosis of 2 hours' duration in the presence of a normal number of leukocytes. Numerous immature, granular leukocytes were present in the peripheral blood during this phase. A completely normal leukocytic blood picture was found 72 hours after the injection and was maintained thereafter.

The number of erythrocytes dropped sharply during the leukocytic phase and returned only gradually within the following 72 hours to the original level. Numerous nucleated erythrocytes (20 to 25 per 100 leukocytes) were present during the erythropenic phase. The volume of packed blood cells and the amount of hemoglobin underwent corresponding changes. The sedimentation rate of the erythrocytes was markedly accelerated during the first 24 hours and gradually returned to a normal level within 6 days. The coagulation time was moderately lengthened during the first hour and became moderately shortened thereafter for several days. The prothrombin time remained within normal limits. The viscosity of the plasma was unchanged, while the viscosity of the whole blood became moderately reduced during the period of erythropenia.

(90 to 115 days) and which were killed at the end of the experiment, or up to 25 days later, invariably showed an excessively enlarged brown-red liver with yellow spotting, varying in weight from 1004 to 1150 gm. The spleen was moderately enlarged, weighing from 55 to 85 gm. The abdominal lymph nodes were also moderately enlarged, while the kidneys were swollen and had a grayish brown color.

Histological examination of the organs showed the following changes attributable to the treatment given: In the three animals (two dogs and one rabbit) which died within the first 21 days of the experiment, the main lesions were located in the liver, which showed a moderate foam-cellular transformation of the parenchymatous cells. It was noted that the marginal areas of the liver cells were homogeneous and very much denser than the central portions, thereby causing a sharp delineation of the liver cell cords from the surrounding distended and edematous sinusoids. The renal glomeruli often exhibited a broadened and homogeneous matrix, while the tubular epithelium was vacuolated or had a foamy appearance. The media of the aorta was loose and edematous; the intima was intact. All other organs (lungs, heart, large arteries, pancreas, spleen, lymph nodes, suprarenal gland, thyroid, parathyroid, brain, hypophysis, intestine, bone marrow) revealed only circulatory changes, such as edema and hemorrhages, or were without any abnormality.

The majority of the organs of the animals subjected to a much more prolonged treatment with gum arabic were normal. In a few dogs the lungs showed small, foam-cellular accumulations in the interstitial tissue and interalveolar septa. The liver was always converted into an organ composed entirely of huge foam cells and often showed pericentral necroses. The foam-cellular transformation also involved the periportal connective tissue as well as the endothelial cells of the hepatic and portal veins (Fig. 1). The spleen presented a similar condition of the pulp, which usually also contained a large number of giant cells of the megakaryocytic type (Fig. 2). The follicles were either normal or showed central hemorrhages. The suprarenal glands contained large masses of foam-cellular reticulum cells in the medulla and not infrequently also in the peripheral cortex (Fig. 3). Foam cells were seen in the reticulum of some of the lymph nodes studied, while others were essentially normal. A moderate number of foam-cellular reticulum cells were present in the bone marrow of one dog. The heart was usually normal. Myocardial arterioles with thickened and hyaline walls were found in one dog. The aortas of all animals showed areas of necrosis and hyalinization of the media, which was also usually calcified, in the region above the aortic valves. The surrounding media was edematous.

were especially numerous when the total leukocytic count was low. There was, moreover, a moderate to marked increase of the immature forms. It is remarkable that during periods when the treatment with gum arabic solution was temporarily interrupted, or during the recovery period following the cessation of treatment, there occurred a moderate leukopenia characterized by a marked decrease of the polymorphonuclear leukocytes. It is furthermore noteworthy that the reported blood changes underwent a relatively rapidly progressing recovery within a few days after the cessation of even a very severe and prolonged treatment. Table III may serve as a representative illustration of the changes in the blood observed after repeated injections.

TABLE III

Changes in the Blood after Repeated Injections of Gum Arabic Solutions

Time	Gum acacia; total amount	Hemoglobin	Erythrocytes	Leukocytes	Polymorphonuclear leukocytes	Sedimentation rate
	(*gm.*)					(*mm.*)
1st day	7.5	15.2	6,350,000	15,400	79	
3rd day	15.0	15.9	6,300,000	13,100	69	1.0
7th day	30.0	16.0	6,520,000	6,800	57	23.0
14th day	68.75	11.8	5,650,000	4,200	80	48.0
18th day	117.50	9.4	3,300,000	1,200	78	45.0
25th day	133.75	8.5	3,650,000	3,300	96	65.0
35th day	133.75	11.9	5,470,000	9,500	82	10.0
55th day	186.75	10.8	4,790,000	6,100	40	29.0
76th day	261.75	8.3	4,140,000	3,600	87	65.0
84th day	301.75	4.5	2,020,000	impossible to count	95	81.0
109th day*		10.7	5,230,000	6,400	53	8.0

* Injections discontinued after 87th day.

Morphological Pathology

The two dogs which died 5 days and 21 days after the start of the experiment showed only a hemorrhagic pneumonia. The other organs were normal in size and appearance. The rabbit which died after 2 weeks of treatment exhibited the same pulmonary pathology in addition to a firm, yellow-brown spleen surpassing by about ten times the size of a normal organ.

The dog which died 40 days after the start of the experiment revealed compression atelectasis of both lungs due to a turbid, reddish fluid containing fibrinous shreds in the pleural cavities. A shallow ulceration, measuring 2 cm. in diameter, was found in the duodenal mucosa about 2 cm. below the pylorus. The liver was huge, weighing 1010 gm., and had a red-yellow mottled surface and a firm consistency. The spleen weighed 48 gm. and was of red color and firm consistency. The other internal organs, including the brain, were normal.

The postmortem examination of the animals which survived longer

The liquid which separated from the beaten whole egg white was injected into the jugular veins of two dogs 5 days a week. The dose given each time was gradually increased from 10 cc. during the first week, to 20 cc. during the next 2 weeks, to 30 cc. during the following 2 weeks, to 40 cc. during the subsequent 3 days, to 50 cc. during the following 5 days, to 65 cc. on the next 2 days, to 80 cc. on the following day, to 90 cc. on the next day, and to 100 cc. on the next 3 days. One dog died directly after the last injection of 65 cc. of egg albumin, while the second dog died immediately after the last of the injections of 100 cc. of egg white. The first dog, weighing 9.06 Kg., received a total of 1060 cc. of egg white, and the second dog, weighing 9.8 Kg., a total of 1330 cc. Several days after the start of the treatment, large amounts of albumin were excreted with the urine, which also contained a few leukocytes and erythrocytes. During the latter part of the experiment the urine contained such large amounts of albumin that upon heating it coagulated to a solid white mass. The head, especially the region of the jaws and eyes, was considerably swollen at times. There were no significant fluctuations in the weights of the two dogs during the 2 months of treatment.

The following tests were made on the blood once a week: amount of hemoglobin, number of erythrocytes and leukocytes, prothrombin time, coagulation time, sedimentation rate, volume of packed erythrocytes, viscosity of whole blood and plasma.

The hemoglobin content remained relatively stationary for the two dogs for the greater part of the experiment and showed a moderate to marked decline only toward the end (12.4 gm. and 14.6 gm., respectively, at the start; 9.5 gm. and 11.6 gm. at the end). Similar fluctuations were observed in regard to the erythrocytes and leukocytes. There was a moderate reduction of erythrocytes and volume of packed blood cells during the second month of the experiment. Prothrombin and coagulation times stayed within normal limits. The sedimentation rate was accelerated to a considerable degree in most tests but returned several times to almost normal values. The viscosity of the whole blood and of the plasma fluctuated within normal limits.

Morphological Pathology

Autopsies of the two dogs showed the spleens to be considerably enlarged, dark red and firm. The livers, kidneys and mucosa of the stomachs and intestines were congested.

Histological examination revealed normal conditions in the lung, pancreas, lymph nodes, heart, adrenal, intestine and stomach. The spleen was considerably congested. The bone marrow was an immature

The intima above these foci was either normal or consisted of a single layer of small foam cells, or foam-cellular accumulations were present in the subintimal space. A foam-cellular polypous formation of the intima of this region was observed once (Figs. 4 to 6). The carotid and femoral arteries were normal. The renal glomeruli were swollen and had thickened capillary walls which seemed to be embedded in a "mucinous" matrix, sometimes obliterating the glomerular structure to a marked extent. Some of the glomerular tufts were adherent to Bowman's capsules, which were occasionally indistinctly outlined against the surrounding tissue. In some glomeruli there was a marked reduction in the number of nuclei, while in others a focal crowding of oval-shaped nuclei was seen (Figs. 7 and 8). The tubular epithelium was often vacuolated or foam-cellular. Small foam-cellular foci were present in the interstitial tissue in a few cases. The walls of the renal arterioles were usually vacuolated, while a thickened and hyaline media existed in one instance. The brain contained scattered perivascular hemorrhages and masses of foam cells in the choroid plexus. Some of the markedly swollen choroid villi lacked an ependymal covering and were infiltrated with lymphocytes.

II. EGG ALBUMIN: FIRST EXPERIMENT

Egg albumin has a macromolecule which is approximately globular and which has a molecular weight of between 34,500 and 43,800, *i.e.*, it is sufficiently small to pass through the glomerular filter at a relatively rapid rate when introduced intravenously (Bott and Richards; Briggs; Bayliss, Kerridge and Russell). In its hydrated form the molecular weight of this protein does not exceed 61,440 (Bull) and thus remains below the critical limit (68,000) of particle size, which permits glomerular filtration. The studies of Manwaring, Marino, McCleave and Boone have shown that egg white does not readily produce an anaphylactic reaction in dogs, thereby permitting the parenteral introduction of relatively large amounts of this substance without the danger of shock.

The albumin obtained from fresh eggs upon separation from the yolks consists of a thin, watery portion and a thicker, viscous portion containing small, opaque, globular particles. A homogeneous mixture of these two components was made by beating the egg white vigorously until a stiff foam was formed. This was kept in the refrigerator for 24 to 48 hours. A slightly yellow, crystal-clear liquid separated out from the foam. This material had a viscosity of 3.3 to 3.5 at room temperature and a pH of 9.3. The viscosity of the thin and watery portion of the egg albumin identically treated was 3.3 at 20° C. and that of the more viscous portion was 3.42.

The intravenous medication was continued during the next 2 days and then stopped. Thus, a total of 230 cc. of 1 per cent amino-antipyrine-azoprotein was administered. Because the dogs showed signs of intoxication, no treatment of any kind was given for 1 week. Subsequently, for a period of 3 months, the dogs received on alternate days, 0.32 gm. of amino-antipyrine orally. As there was no appreciable effect upon the status of the leukocytes, no further treatment was given; the dogs were killed approximately 2 months after the last medication of amino-antipyrine.

Morphological Pathology

The autopsies showed small kidneys with numerous cicatricial cortical retractions. The spleens were small, dark red and had granular surfaces.

Upon histological examination the walls of some of the myocardial arterioles were found to be in part bluish-stained; others had thickened hyaline walls lined by a proliferated and clumped endothelium. The majority of the coronary arteries, however, were normal. The myocardium was without any abnormalities. The liver cells had a fine vacuolar or foamy cytoplasm. The adrenals were normal. The spleen was congested. Some amorphous brown pigment was located within the cytoplasm of large, reticular foam cells, which were found in focal accumulations or scattered singly throughout the splenic pulp. Their cytoplasm stained a grayish blue. The spermatogenic epithelium of the testes was highly atrophic. The bone marrow of the sternum was a myeloid fat tissue containing numerous mature cells.

The kidneys showed in the cortex localized mononuclear and foam-cellular infiltrations and wedge-shaped areas of fibrous scar tissue. The afferent arterioles and the pedicles of the glomeruli often had thickened hyaline or very cellular walls. The capillary loops were frequently thickened and hyaline, converting the glomerulus into an irregular, coarse network of hyaline bands in which irregularly shaped nuclei were found. The capillary lumina of such glomeruli were distended and sometimes cystic. In others there was an almost complete obliteration of the capillary structure by the swollen and coalescing, hyaline, vascular structures. In some of the cystic glomerular cavities foam cells were found or large, syncytial, bluish-stained masses with numerous, clumped, pale-stained nuclei (Fig. 12). The hyalinizing processes sometimes involved only a part of a glomerulus. The walls of some of the medium-sized arteries were highly vacuolated, while the walls of the arterioles showed a moderate to marked hyaline thickening with swelling and foam-cellular transformation of the endothelium leading

yeloid tissue. The liver was highly edematous and congested. The er cell strands were separated by distended sinusoids filled and sur-unded by an albuminous material. The liver cells were mildly vacuo-ed, or small and atrophic. The Kupffer cells frequently contained an morphous brown pigment. The kidneys of one of the dogs contained merous wedge-shaped, scarred areas in the cortex composed of und cells, distended tubules with ill-defined epithelial lining and with stringy, albuminous content, indistinctly outlined multinucleated ant cells in the interstitial fibrous tissue, and arterioles with thickened d hyaline walls and narrowed lumina (Fig. 9). The glomeruli within ese areas, as well as in the surrounding tissue, were rich in nuclei and owed thickened capsules. The tubules contained hyaline casts and owed a marked vacuolization and degeneration of the epithelial lin-g. The kidney of the second dog had distended tubular lumina filled th a stringy, albuminous material and lined by small epithelial cells by highly vacuolated, swollen cells. The glomeruli were atrophic and nall, while the capsular spaces were dilated and filled with an album-ous matter. The walls of the arterioles were swollen, edematous and acuolated. The renal arteries in both dogs were normal.

The ascending aorta of one dog showed a large hyaline area with crosis and central calcification, situated in the outer media. There ere focal granular and diffuse calcific deposits in the inner media thin subintimal, hyaline scar tissue in the ascending aorta of the cond dog (Fig. 10). The endothelium of this aorta had proliferated d showed palisade arrangement elsewhere. The coronary arteries d highly edematous walls and showed distortion of their normal ructure. A small hyaline intimal thickening was found in one of the nall abdominal arteries (Fig. 11).

EGG ALBUMIN: SECOND EXPERIMENT

In a second experiment, egg albumin was combined by diazotization, ing the method of Landsteiner, with amino-antipyrine, producing ereby an amino-antipyrine-azoprotein. The original purpose of this periment was the experimental production of an agranulocytosis on e basis of a specific allergy, through sensitization of dogs by repeated travenous injections of a 1 per cent solution of this azoprotein. While is goal was not achieved, significant vascular changes were observed.

Two dogs, both weighing approximately 10 Kg., were used. They ceived 10 cc. of a 1 per cent solution of amino-antipyrine-azoprotein to the jugular vein five times a week. After 21 injections had been ven within 3 weeks, the treatment was discontinued for 2 days, and 32 gm. of amino-antipyrine was given by mouth on 4 successive days.

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Two dogs, both weighing approximately 10 Kg., were used. They received 10 cc. of a 1 per cent solution of amino-antipyrine-azoprotein into the jugular vein five times a week. After 21 injections had been given within 3 weeks, the treatment was discontinued for 2 days, and 0.32 gm. of amino-antipyrine was given by mouth on 4 successive days.

were given. The dose was then increased to 15 cc., 20 cc. and 40 cc., on successive days. After 3 injections of 40 cc. the dose was increased for each of the following 2 injections to 60 cc. and 65 cc. After 1 injection of 80 cc. and 1 of 100 cc. the daily dose was elevated to 120 cc. for the next 5 injections. For the subsequent 4 administrations of 120 cc. the concentration of the gelatin solution was increased from 17 per cent to 25 per cent. No further treatment was given to two dogs which were killed 4 weeks after the last injection. In two other dogs of this series the treatment was resumed after 4 weeks for a period of 6 days, during which 120 cc. of a 25 per cent solution was injected each day. Two days after the last injection these two dogs were killed. The fifth dog in this series was killed after it had received 2 injections of 10 cc. each of a 10 per cent solution, 3 injections of 10 cc. and 1 injection of 15 cc. of a 17 per cent solution, as it showed signs of severe dyspnea. This dog received within 8 days, during 5 injections, 65 cc. of gelatin solution containing a total of 9.65 gm. of dry gelatin. Two dogs received within 2 months, 1840 cc. of gelatin solution containing 414 gm. of gelatin in 27 injections, and two other dogs, in 39 injections, received 2,849 cc. of gelatin solution containing a total of 576 gm. of gelatin within 11 weeks.

Apart from a somewhat increased salivation observed directly after the injection of the larger doses of gelatin solution, there were no symptomatic reactions of any kind. The urinalysis made at irregular intervals showed only traces of albumin and an occasional leukocyte and erythrocyte in the sediment.

The same tests of the blood as in the study of the effect of egg albumin were made at weekly intervals. The amount of hemoglobin, the number of erythrocytes and the volume of packed red blood cells decreased considerably with the progressive injection of gelatin solution, but underwent a relatively rapid recovery after cessation of treatment. At the same time the sedimentation rate increased rapidly and considerably. The leukocytes, prothrombin time and coagulation time fluctuated markedly but remained within the extremes of the normal range. The viscosity of the whole blood and especially that of the plasma became appreciably increased beyond the normal limit, particularly during the final part of the experiment, when large doses of a 25 per cent gelatin solution were given. Table IV, giving the data obtained in dog no. 724, weighing 5.6 Kg., is a representative example of the observations made.

In view of the fact that the increased viscosity of the blood and plasma indicated the retention of gelatin in the blood, an attempt was made to demonstrate the presence of this material by an indirect

to an almost complete occlusion of the lumina. The tubular epithelium was essentially normal.

The intima of the aorta occasionally contained small foam cells, which invaded the superficial layers of the underlying edematous media (Fig. 13). In other parts there were a few large hyaline scars in the media of the aorta. In sections of some of the large elastic arteries there was found an extreme cushionlike, fibrous thickening of the intima. Groups of small foam cells were scattered through these fibrous and partly hyalinized thickenings and were situated mainly near the media (Fig. 14). Smaller cushions consisted almost exclusively of foam-cellular proliferations of the intima.

III. GELATIN

Gelatin, the water-soluble derivative of collagen, varies considerably in its molecular weight (35,000 to 150,000), depending upon the method of preparation. It is for this reason that only those portions and types of gelatin having a molecular weight below 68,000 when hydrated will pass through the glomerular filter, while the larger molecular types of gelatin are retained at least temporarily in the body after parenteral introduction. While impure gelatin gives rise to the development of allergic and toxic reactions (Taylor and Waters), it is usually contended that purified gelatin does not possess allergenic properties, because cyclic amino acids are absent in its molecule.

A high-grade gelatin (*Coignet Père and Fils, Silver Label*) was dissolved over a steam bath in a 1 per cent sodium chloride solution. The hot solution was mixed with a large amount of "Hyflo-Supercel" and filtered through Whatman no. 1 filter paper in a Büchner funnel, using strong suction. The filtered solution, which was brown and slightly cloudy, was then autoclaved at 15 lbs. for 20 minutes.

A 1 per cent solution thus prepared has, at 24° C., a viscosity of 1.38; a 2 per cent solution has a viscosity of 1.88, or approximately that of normal human and canine plasma. A 17 per cent solution has a pH of 5.25.

Five dogs were employed in the experiment. Four of them were injected intravenously, while the fifth dog, for the greater part of the experiment, was injected intraperitoneally after it became difficult, following 6 intravenous injections, to enter the jugular vein because of the extremely short neck of this animal. The weight of the dogs ranged from 5.6 to 12.37 Kg. The injections were given five times weekly. The concentration as well as the dose of gelatin solution administered was gradually increased. After 2 injections of 10 cc. of a 10 per cent solution, 3 injections of 10 cc. of a 17 per cent solution

brain, pancreas, spleen, lymph nodes, bone marrow and intestines were without abnormalities. The liver was edematous and contained moderately atrophic or vacuolated cells. The myocardium was normal, while the coronary arterioles showed swollen, vacuolated and edematous walls with focal hyalinization in some instances (Fig. 15). The aorta and the large and medium-sized arteries of the abdomen revealed moderate to marked medial edema in two dogs. In addition to similar edematous changes there were subintimal and medial calcification and hyalinization in the arteries of the other three dogs of this series. The intimal hyaline and fibrous thickenings were very extensive in the aorta of the dog which was injected intraperitoneally (Fig. 16). The calcification found in this case involved not only areas of medial necrosis but also the internal elastic membrane (Fig. 17). A smaller artery showed a dissecting aneurysm destroying the media in large areas. While the kidneys of two dogs were essentially normal, in the other three dogs these organs showed a marked vacuolization of the tubular epithelium. The walls of the renal arteries and arterioles were vacuolated and edematous, and medial hyalinization and intimal hyaline thickenings were found in some instances. Some of the arterioles were occluded by hyaline masses infiltrating the walls and containing irregularly arranged and shaped nuclei (Fig. 18). Small polypous endothelial proliferations were seen in the branches of the renal artery. The glomerular capillaries in the dog injected intraperitoneally were distended and showed a marked, sometimes networklike, proliferation of the endothelial cells. The glomeruli were swollen and congested and erythrocytes were present in some of the Bowman's capsular spaces of this dog.

Comment

The observations presented show that the intravenous injection of solutions of gum arabic, ovalbumin and gelatin elicit in the blood a complex of cellular and plasmatic reactions characteristic of macromolecular hematic disturbances and similar to those caused by the intravenous introduction of polyvinyl alcohol, methyl cellulose and pectin; *i.e.*, substances used or proposed as nonhematogenous blood substitutes. The data recorded indicate that the protracted phase of this response, following upon the repeated administration of the substances studied, is in general less pronounced than that seen with polyvinyl alcohol and methyl cellulose and resembles more closely that found with pectin.

It is remarkable that even after a very intensive treatment with large amounts of highly viscous solutions of gum arabic and gelatin,

method. Twenty-four hours after the dogs had received five injections, during 5 days, of 120 cc. of the 25 per cent gelatin solution, blood was withdrawn and mixed with sufficient amounts of potassium oxalate to prevent clotting. This blood solidified when placed in the refrigerator and became liquid again when heated to body temperature. Plasma from a normal dog and from the animals injected with gelatin was

TABLE IV

Blood Reactions during Repeated and Massive Intravenous Administration of Gelatin Solution

Time	Gelatin	Hemoglobin	Erythrocytes	Volume packed erythrocytes	Sedimentation rate	Leukocytes	Viscosity	
							Whole blood	Plasma
	(*gm.*)			(*mm.*)	(*mm.*)			
1st day		13.8	6,630,000	44.5	1.0	15,100	4.76 at 22.2° C.	1.93 at 22.5°C.
6th day	2.0	12.5	5,910,000	41.0	32.0	20,300	5.48 at 19.2°	2.16 at 21.0°
16th day	23.25	12.1	6,270,000	41.0	45.0	25,800	5.06 at 19.0	2.05 at 21.1°
22nd day	41.95	10.5	5,010,000	36.0	55.0	16,500	4.10 at 21.8°	2.30 at 21.8°
30th day	77.65	11.8	6,000,000	35.0	58.0	14,500	4.76 at 23.4°	2.35 at 19.4°
36th day	108.25	10.3	5,060,000	32.0	61.0	15,100	4.66 at 21.5°	2.36 at 19.6°
43rd day	150.75	10.9	5,690,000	29.5	58.0	16,100	5.70 at 20.0°	2.72 at 18.5°
51st day	253.60	8.0	3,770,000	28.0	58.0	15,300	8.10 at 22.3°	4.3 at 19.3°
57th day	324.40	8.3	4,480,000	29.0	69.0	20,500	6.04 at 22.0°	3.80 at 21.1°
63rd day	414.40	5.9	3,500,000	23.0	62.0	14,000	4.10 at 21.4°	2.68 at 20.0°

then heated in test tubes in a water bath to a temperature of 95° C. for 5 minutes, when the proteins were well coagulated. The coagulum was then broken up by a wire, and the tubes, while hot, were centrifuged for 30 minutes. About 1.5 cc. of a liquid material was expressed thereby from an original amount of 3 cc. of plasma. This expressed liquid was clear and pale straw-colored. When the fluid expressed from the coagulated normal plasma was placed in the refrigerator, it remained liquid, while that obtained from the plasma of the animals injected with gelatin solidified, showing thereby that it was composed in part of gelatin.

Morphological Pathology

Autopsies of the dogs showed essentially normal organs upon gross examination. The spleens were in general moderately enlarged, dark red and semifirm.

Histological examination showed that the lungs, pituitary body,

brain, pancreas, spleen, lymph nodes, bone marrow and intestines were without abnormalities. The liver was edematous and contained moderately atrophic or vacuolated cells. The myocardium was normal, while the coronary arterioles showed swollen, vacuolated and edematous walls with focal hyalinization in some instances (Fig. 15). The aorta and the large and medium-sized arteries of the abdomen revealed moderate to marked medial edema in two dogs. In addition to similar edematous changes there were subintimal and medial calcification and hyalinization in the arteries of the other three dogs of this series. The intimal hyaline and fibrous thickenings were very extensive in the aorta of the dog which was injected intraperitoneally (Fig. 16). The calcification found in this case involved not only areas of medial necrosis but also the internal elastic membrane (Fig. 17). A smaller artery showed a dissecting aneurysm destroying the media in large areas. While the kidneys of two dogs were essentially normal, in the other three dogs these organs showed a marked vacuolization of the tubular epithelium. The walls of the renal arteries and arterioles were vacuolated and edematous, and medial hyalinization and intimal hyaline thickenings were found in some instances. Some of the arterioles were occluded by hyaline masses infiltrating the walls and containing irregularly arranged and shaped nuclei (Fig. 18). Small polypous endothelial proliferations were seen in the branches of the renal artery. The glomerular capillaries in the dog injected intraperitoneally were distended and showed a marked, sometimes networklike, proliferation of the endothelial cells. The glomeruli were swollen and congested and erythrocytes were present in some of the Bowman's capsular spaces of this dog.

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In view of the thesis advanced concerning the causal relationship between hematic colloidal disturbances and arteriosclerotic or atheromatous lesions, it is significant that the physicochemical macromolecular changes produced in the plasma of the animals treated with gum arabic, gelatin and ovalbumin are accompanied by the appearance of degenerative and proliferative reactions in arterial walls. It is noteworthy that the arterial and arteriolar lesions present in the animals injected with the two foreign proteins are predominantly of the sclerosing type; *i.e.*, they consist of marked fibrous intimal thickening and medial hyalinization and necrosis and are not of the atheromatous character.

This response of the arterial, and especially the intimal, tissues to the presence of foreign proteins and their metabolic or allergic reaction products seems to be in accord with the chemical nature of these substances, as their inclusion into the cytoplasmic matter of the reacting intimal cells cannot lead to the primary formation of foam cells. Such an event under existing experimental conditions is either the result of a secondary cellular degeneration or represents the product of an excessive cytoplasmic hydration.

The arterial lesions elicited in these dogs are comparable in type and causative mechanism to the arteriosclerotic changes seen by Dick and Leiter in rabbits in connection with the hyperglobulinemia and amyloidosis following the repeated injection of streptococcic cultures. They are also similar to the arterial lesions described by Holman in dogs subjected to repeated intravenous injection of large amounts of altered plasma and attributed to an allergic reaction. However, they differ in morphological type, as well as in chemical nature of the causal substances involved, from the arterial lesions elicited in rabbits by disturbances of protein metabolism, as rabbits kept on a diet rich in proteins develop a hypercholesterolemia and cholesterol atheromatosis (Clarkson and Newburgh; Meeker and Kesten).

The foam-cellular, atheromatqus changes observed in the animals treated with gum arabic represent, on the other hand, counterparts to the lesions elicited by hypercholesterolemia, polyvinylosis and methyl cellulosis. It appears likely that the relatively moderate degree of these changes in animals treated with gum arabic, when compared with those in animals treated with cholesterol, polyvinyl alcohol or methyl cellulose, is attributable to the instability of the macromolecules of gum arabic and the resulting fleeting nature of the disturbances in the colloidal plasmatic equilibrium.

It is probable, on the other hand, that the mechanism in the production of the arterial lesions in chronic arabinosis is identical with

there develops only a relatively minor increase in the viscosity of the plasma, an effect which contrasts sharply with the marked and sustained elevations in plasmatic viscosity obtained by the injection of solutions of polyvinyl alcohol and methyl cellulose. The gelation reaction of the plasma in animals injected with gelatin as well as the physicochemical plasmatic disturbances and the presence of a massive granulation of extraneous matter in the plasma of the dogs injected with gum arabic are, on the other hand, satisfactory proof that the blood contains considerable amounts of the injected foreign material. These findings suggest that the macromolecules of these substances undergo in the blood a certain amount of degradation resulting in a reduction of the viscosity of their colloidal solution. This observation is important for therapeutic reasons, as the size of the molecule controls the degree of retention of these substances in the blood and thus also the magnitude and duration of the colloidal osmotic pressure exerted by them.

The behavior of gum arabic and gelatin is explainable by the fact that gum arabic is relatively unstable and tends to become partially hydrolyzed, while gelatin is readily degraded by the proteolytic enzymes present in the body into its amino acid constituents, which are used in the normal metabolism. It is apparently for these reasons that gelatin, parenterally introduced, does not give rise to appreciable phenomena, which form a typical and remarkable feature of the organic lesions elicited by gum arabic. Both substances, however, seem to remain sufficiently long in a macromolecular state in the blood to cause degenerative changes in the glomerular filtration membranes.

Somewhat different conditions apparently prevail in these respects in regard to the injected egg albumin. The observations reported by Briggs, as well as the renal and urinary findings recorded here, indicate that a large portion of the ovalbumin intravenously introduced is rapidly excreted through the kidneys. However, only a part of the albumin demonstrable in the urine under such conditions represents egg albumin, while another portion is serum albumin to which the glomerular filter becomes permeable following the passage of the foreign protein (Briggs). Moreover, this investigator pointed out that a part of the injected ovalbumin remains in the organism and is present in the blood over prolonged periods. The glomerular lesions found in the dogs treated with this protein suggest that ovalbumin or complexes formed by it may clog the glomerular capillaries and lead to a distension of their lumina, to the development of hyaline changes and to a proliferation of endothelial cells and giant cells (Welker, Andrews and Thomas). It is noteworthy that the renal lesions were particularly severe in the dogs injected with the allergenic azoprotein.

to capacity with foreign matter and separated from their normal connection possess such extraordinarily aggressive qualities and prolonged survival rate. It is known that cells of similar derivation, such as megakaryocytes, released into the blood under abnormal conditions, undergo rather rapid disintegration in the lumina of the pulmonary capillaries.

It is, moreover, a pure assumption that lipophagic foam cells possess a special attraction to the arterial wall, and that for this reason the resulting atheroma represents the normal graveyard of these cells. There can be no doubt that foam cells containing other substances (polyvinyl alcohol, methyl cellulose, pectin, gum arabic) do not exhibit such an affinity. Yet huge, foam-cellular mononuclear cells can be readily demonstrated in the blood smears of dogs treated with methyl cellulose.

Conclusions

The intravenous injection of colloidal solutions of gum arabic, gelatin and ovalbumin elicits in the blood of dogs and rabbits responses which are characteristic of the "macromolecular hematological syndrome" (reduction of erythrocytes and hemoglobin, hastened sedimentation of erythrocytes, transitory leukopenia, persistent leukocytosis or leukopenia and reduction of serum proteins) and represent, in part, expression of the disturbances produced in the equilibrium of the colloidally dispersed macromolecules of the plasma.

The development of storage phenomena in the internal organs depends upon the stability of the injected macromolecules. The injection of gelatin and ovalbumin therefore does not give rise to a thesaurosis, while gum arabic, which cannot be readily and completely depolymerized in the body, is retained in the liver cells and in the reticuloendothelial cells and histiocytes of this organ and of the kidney, spleen, lymph nodes and adrenal.

All three substances apparently cause a clogging of the glomerular filtration membranes when present in excessive amounts in the blood, and thereby elicit degenerative and proliferative changes in the glomeruli.

The injected foreign proteins produce degenerative and proliferative lesions in the elastic and muscular arteries which are of the sclerosing type, while gum arabic causes the development of atheromatous responses. The chemical type and the physicochemical properties of the causative macromolecular agent thus determine the morphological character of the ensuing arterial reactions.

The agents, such as macromolecular carbohydrates (polyvinyl alco-

that in atheromatosis associated with cholesterolosis, polyvinylosis and methyl cellulosis. Christie, Phatak and Olney have demonstrated that gum arabic solution injected intravenously interferes with the oxygenation of erythrocytes. Previous studies (Martin and Hueper) have shown that the blood of dogs given polyvinyl alcohol and of rabbits given cholesterol exhibits a similar impairment of the oxygenation speed of the erythrocytes, probably by the formation of a film of these substances on the surface of the cells. It is postulated that a similar film on the surface of the intima of vessels exerts a corresponding effect upon the exchange of essential metabolites and thus produces a defective oxygenation and nutrition of the arterial walls. In view of the fact that the cholesterol level of the blood is lowered in animals injected with gum arabic, it is unlikely that cholesterol plays any significant part in the causation of the arterial changes.

Leary recently contended that cholesterol atheromatosis in man and rabbits is caused by the active invasion of the arterial walls by lipoidal, foam-cellular phagocytes circulating in the blood and originating from desquamated Kupffer cells and reticulum cells of the adrenal. These cells are said to take up excess cholesterol esters, to possess an extraordinary stickiness after their escape into the blood stream and to have a special, although unexplained, affinity for the tissues of the vascular wall, where they lead a prolonged existence and stimulate the growth of connective tissue.

The present observations with gum arabic indicate that in spite of an extensive transformation of the reticulo-endothelial cells of the liver and adrenal and of other histiocytes into foam-cellular elements, there occurred only moderate atheromatous formation. While attempts at demonstrating foam cells in the numerous blood smears taken during the course of the experiment were unsuccessful, it cannot justly be assumed that these reticulo-endothelial cells, swollen with gum arabic, do not desquamate and do not circulate in the blood. Histological studies did not succeed in demonstrating in a single instance in these and previous experiments the presence of a thrombuslike attachment of foam cells to the intima of any vessel. Moreover, it appears unlikely that such huge foam cells should stick to a much higher degree to the intimal surface of large vessels with their swift blood current than to the wall of small vessels and capillaries, in the lumina of which these cells become temporarily or permanently arrested. There was, on the other hand, a direct foam-cellular transformation of the intimal lining of the aorta, similar to that seen frequently in the vessels of various species treated with polyvinyl alcohol, methyl cellulose or cholesterol. It may be doubted, furthermore, that reticulo-endothelial cells loaded

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hol, pectin, methyl cellulose, gum arabic) and lipoids (cholesterol), which form emulsions with proteinic solutions, give rise to foam-cellular atheromatous lesions.

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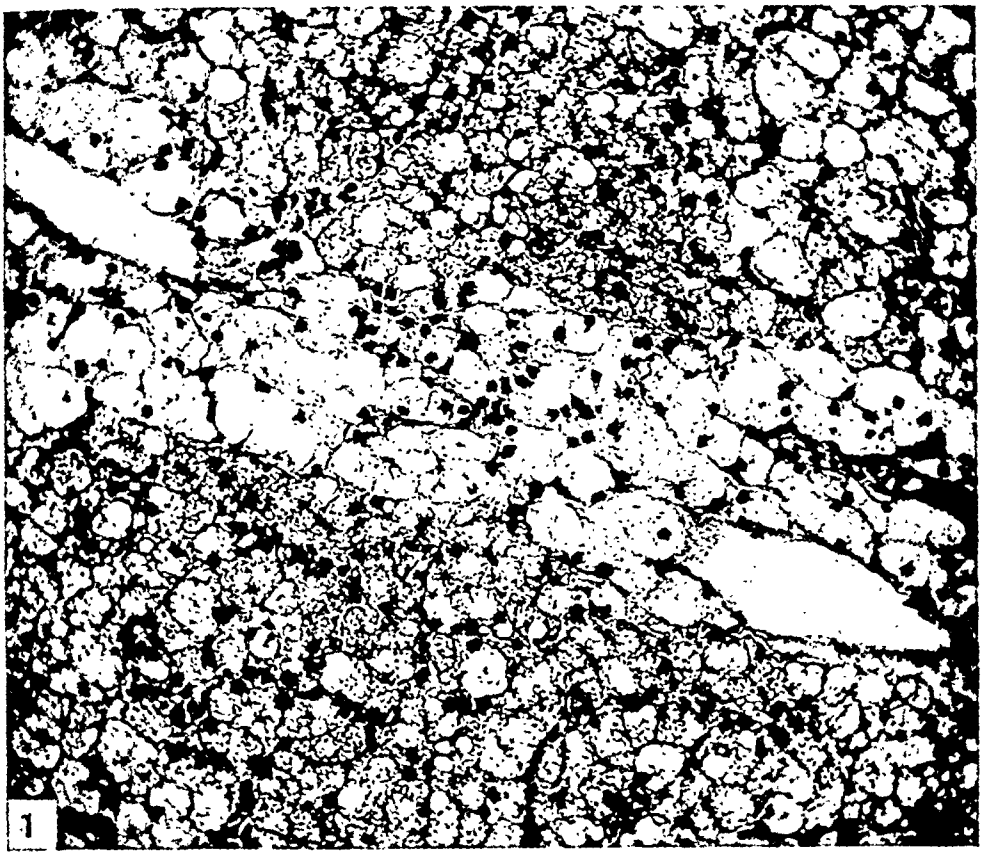
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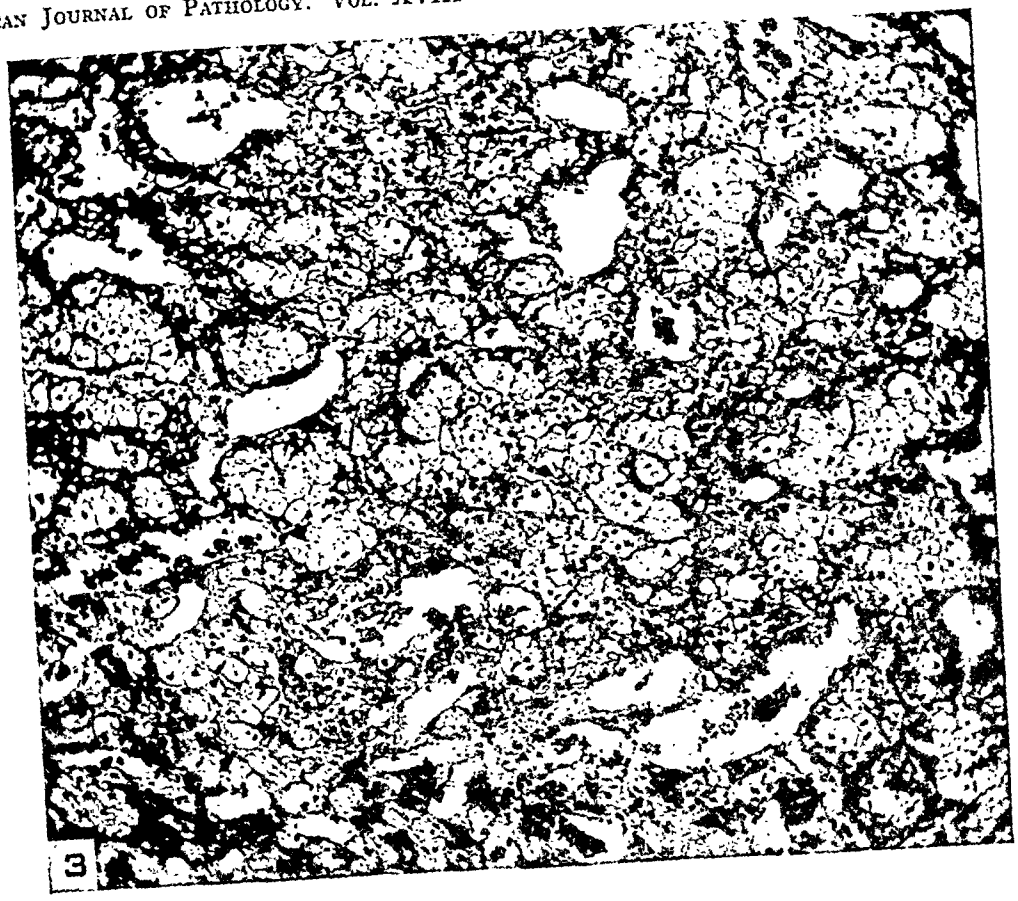
DESCRIPTION OF PLATES

PLATE 133

FIG. 1. Hepatic veins lined in part by foam cells and surrounded by a strand of light-colored connective tissue foam cells, on both sides of which foam-cellular liver cells are found. × 230.

FIG. 2. Foam-cellular splenic pulp with several megakaryocytic giant cells. × 230.

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3



4

Intravenous Injections of Macromolecular Substance

Hueper

PLATE 134

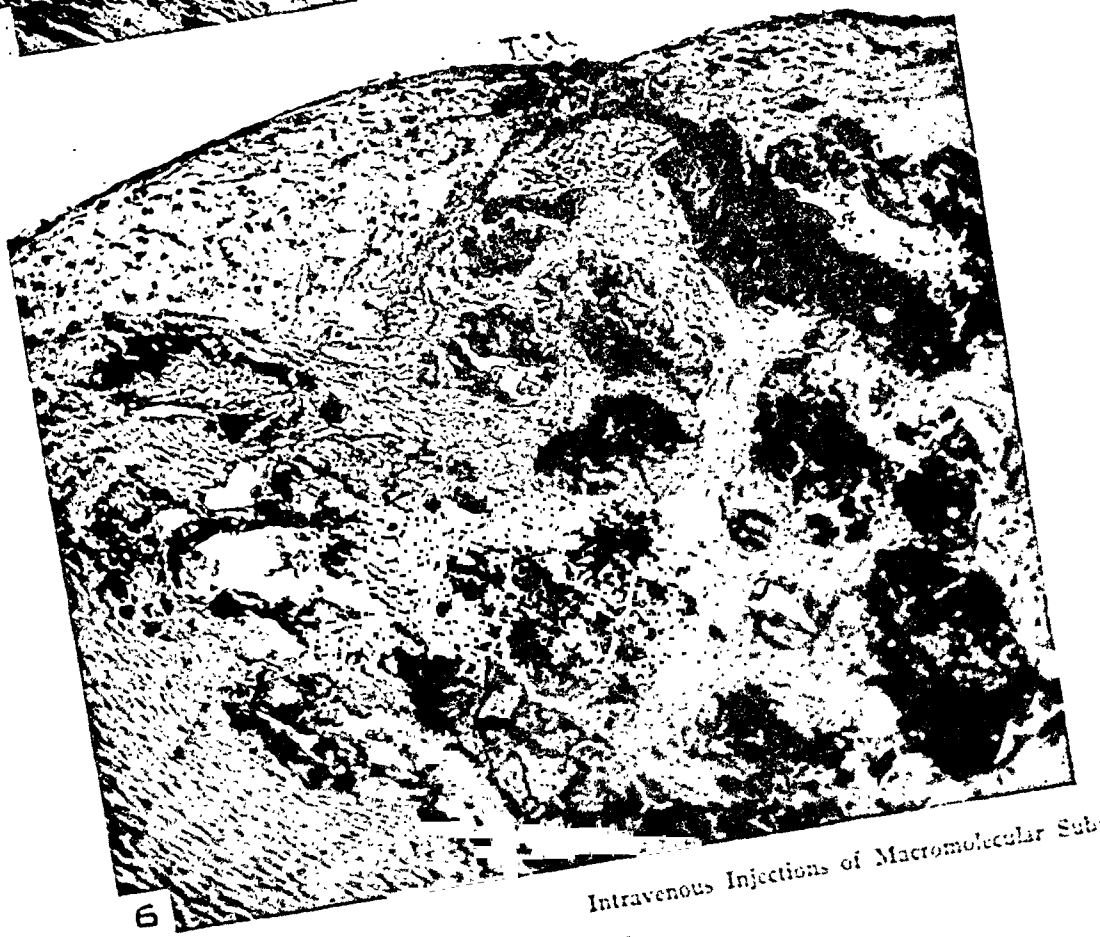
FIG. 3. Adrenal medulla with diffuse foam-cellular transformation of the reticulum cells. × 110.

FIG. 4. Foam-cellular transformation of the intima of the aorta with foam-cellular infiltration of the inner parts of the media. × 230.

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6

Intravenous Injections of Macromolecular Substances

PLATE 135

FIG. 5. Foam-cellular intimal thickening of the aorta with an underlying necrosis of the media. × 150.

FIG. 6. Large calcified necrosis in the media of the aorta with a small foam-cellular accumulation in the subintima. × 120.

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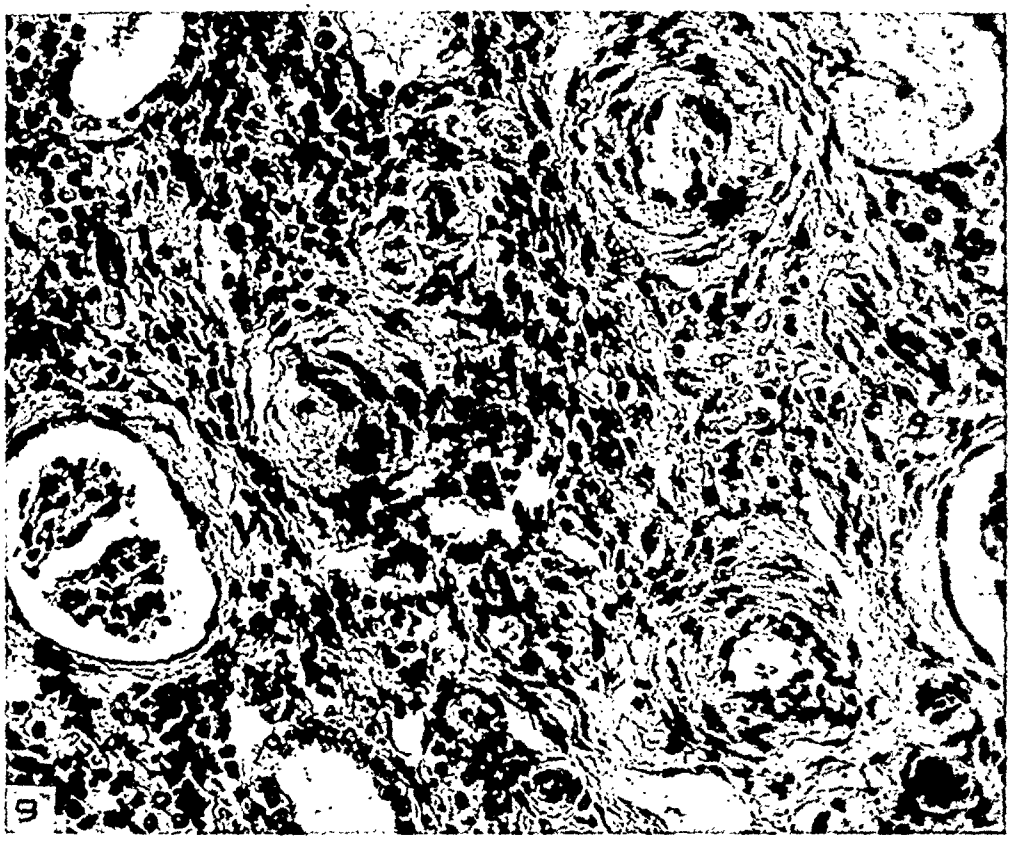
8

Intravenous Injections of Macromolecular Substances

PLATE 136

FIG. 7. Glomerulus in the kidney of a dog with (mucohyaline) thickening of the glomerular framework and a proliferation of the endothelial cells. × 425.

FIG. 8. Glomeruli in the kidney of a rabbit with multicystic swelling of the capillary loops and accumulation of foam cells in the glomeruli and in the surrounding interstitial tissue. × 260.





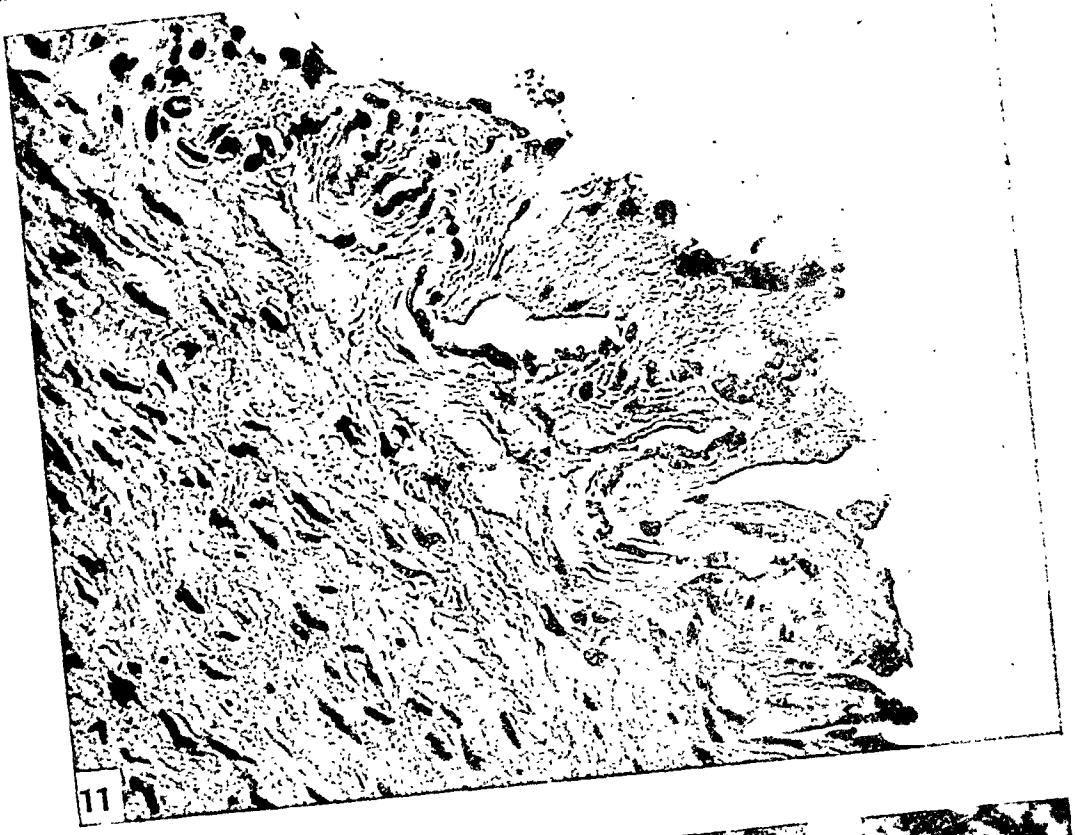
Hueper Intravenous Injections of Macromolecular Substances

PLATE 137

FIG. 9. Renal arterioles with thickened walls and proliferated endothelium embedded in a fibrous stroma infiltrated with lymphocytes. × 230.

FIG. 10. Wall of ascending aorta with hyaline medial necrosis showing local calcification. × 230.

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12

Intravenous Injections of Macromolecular Substances

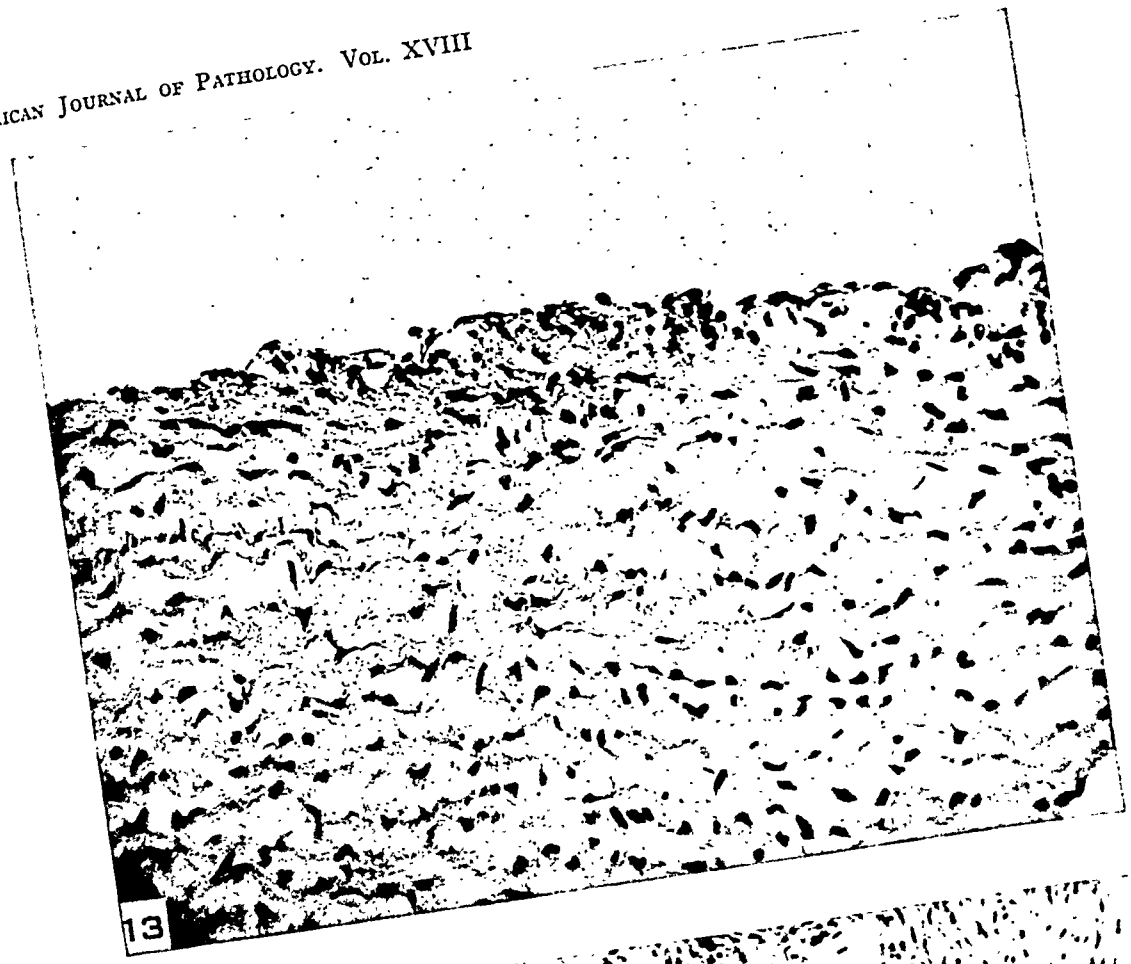
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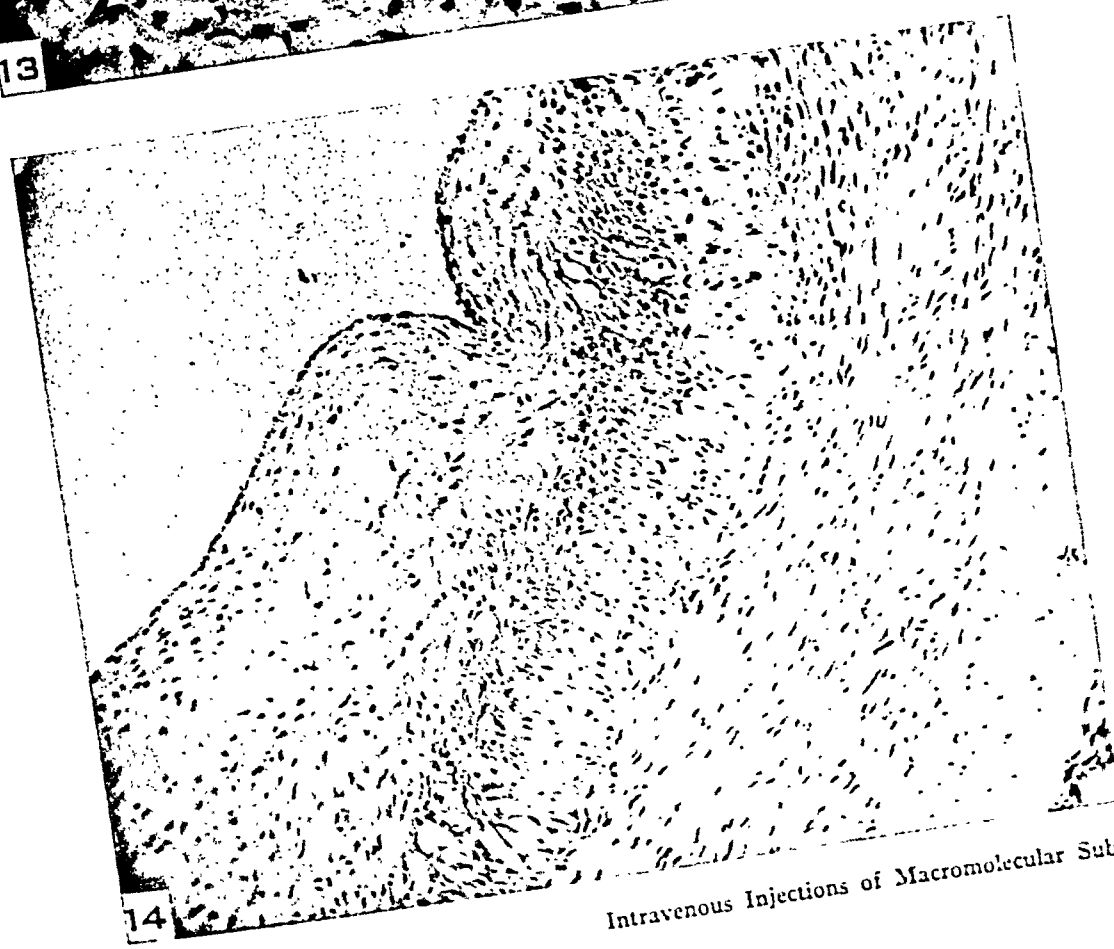
PLATE 138

FIG. 11. Small abdominal branch of aorta with localized hyaline intimal thickening. × 350.

FIG. 12. Glomerulus with multinucleated, syncytial mass and hydropic swelling of the capillary network. × 425.

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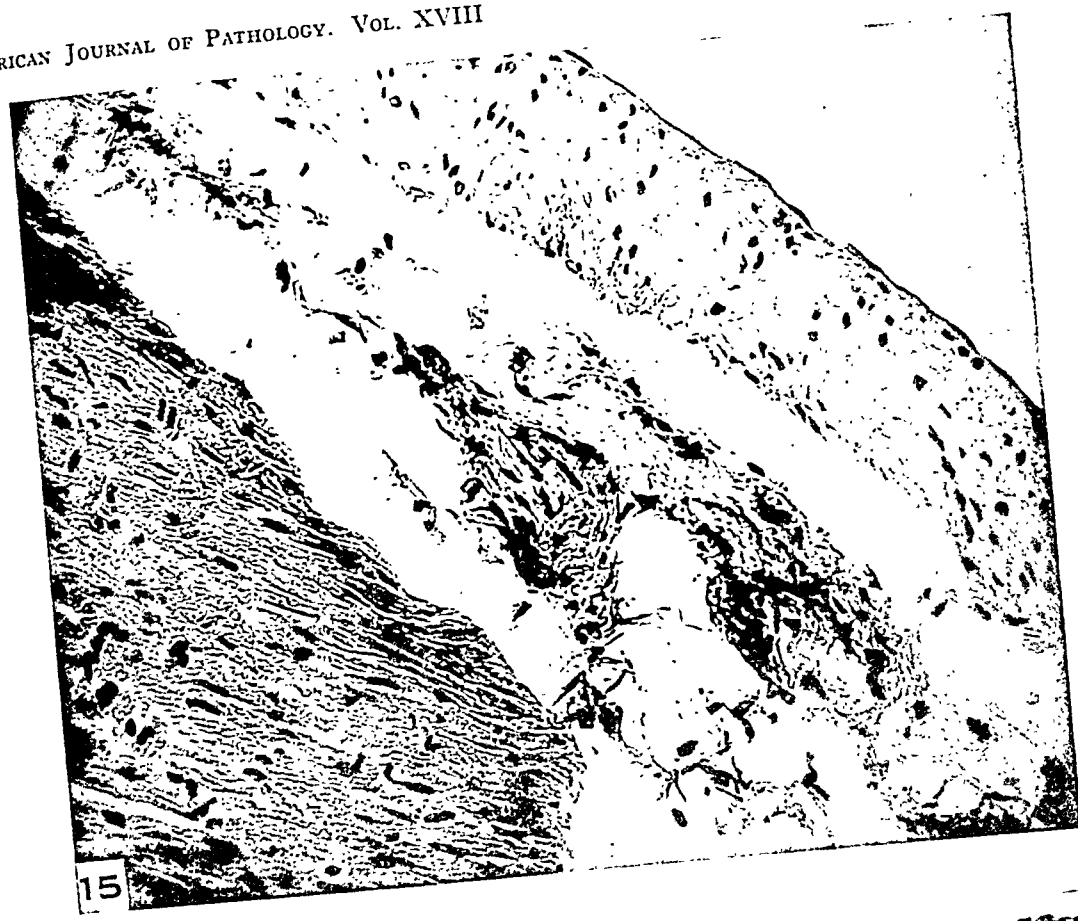
Intravenous Injections of Macromolecular Substances

PLATE 139

FIG. 13. Edematous wall of aorta with hydropic mononuclear cells underneath the intima. × 230.

FIG. 14. Thick cushionlike fibrous intimal thickening of aorta with foam-cellular elements in its basal portion. × 125.

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Intravenous Injections of Macromolecular Substances

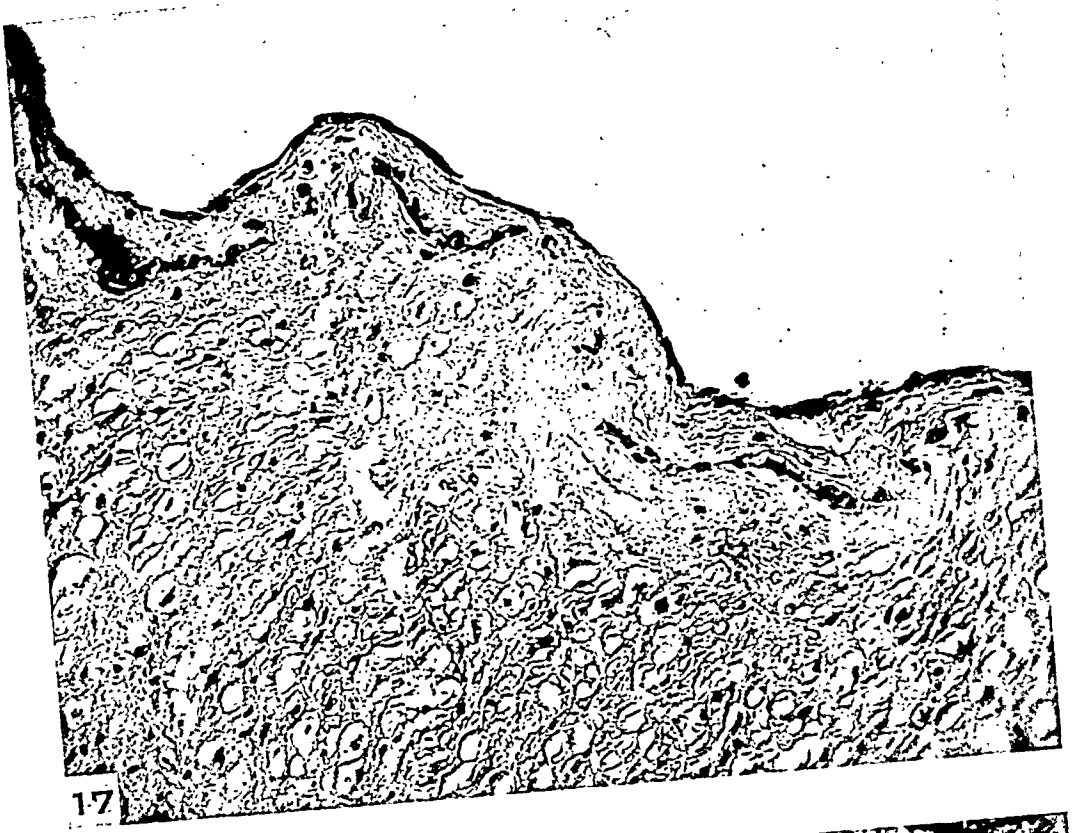
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PLATE 140

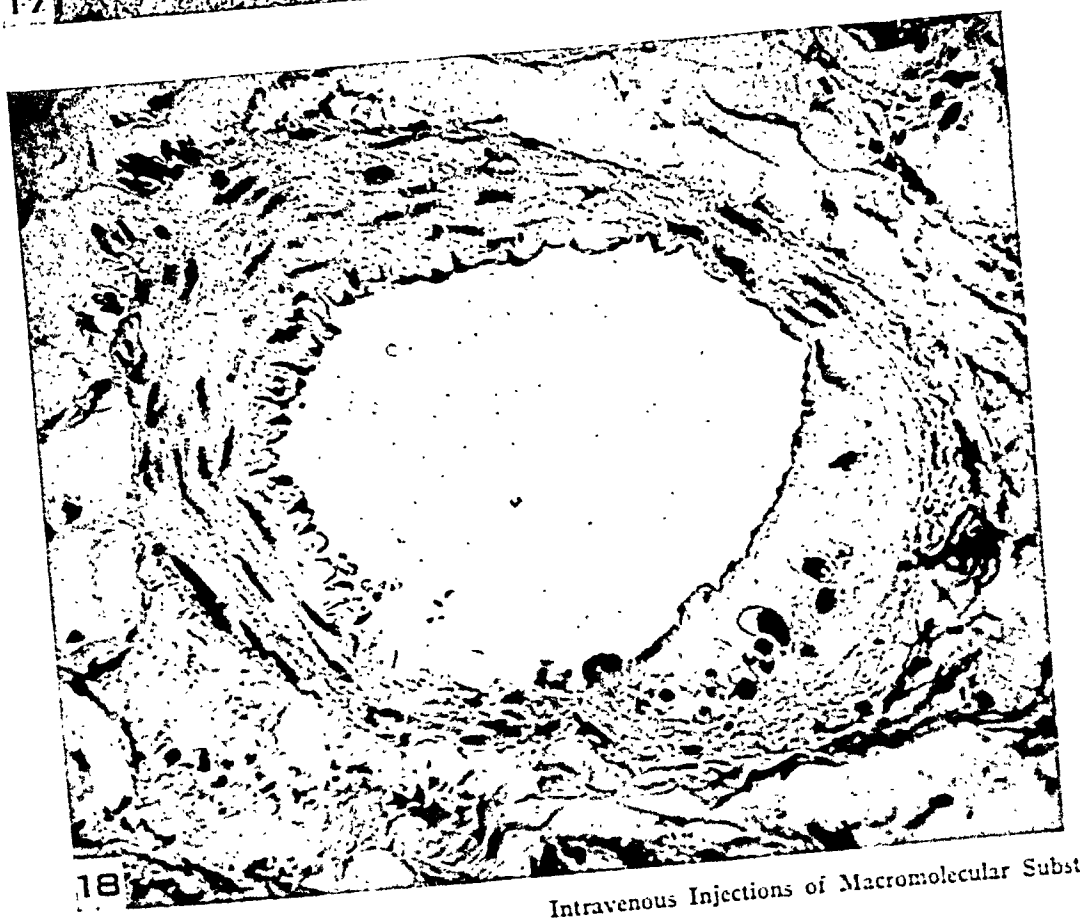
FIG. 15. Myocardial artery with partly hyalinized and vacuolated media. × 175.

FIG. 16. Extensive medial hyalinization in the aorta. × 110.

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Intravenous Injections of Macromolecular Substances

Hueper

PLATE 141

FIG. 17. Intimal hyaline thickening of aorta with calcification of internal elastic membrane. × 150.

FIG. 18. Renal arteriole with hyalinization and swelling of wall. × 230.

SPONTANEOUS CEREBELLAR HEMORRHAGE*

Report of Fifteen Cases

Nathan Mitchell, M.D., and Alfred Angrist, M.D.
(From the Department of Pathology, Queens General Hospital, Jamaica, N.Y.)

Beginning with the 3 cases described by Morgagni and Lieutard and cited by Sédillot in 1813,[1] there has been a total of 109 cases of non-traumatic cerebellar hemorrhage reported in the literature. This group of cases will be designated as *series A*. From November, 1935 to November, 1941, 3,881 autopsies were performed in the Department of Pathology at Queens General Hospital. During this period, a total of 115 spontaneous intracranial hemorrhages were observed. Of these, 100 were located in the cerebrum and/or brain stem, while 15 were found in the cerebellum proper. The latter 15 instances will be referred to hereafter as *series B*. All cases of cerebral thrombosis with softening, old hemorrhages of the cerebrum and the cerebellum and traumatic intracerebral and intracerebellar hemorrhage are excluded from consideration.

Reports of Cases

Case 1

F. T. (A-40-266M), a white female, 65 years old, suddenly became comatose 8 hours before admission. For a few months purpuric spots had been noted in the skin associated with firm subcutaneous masses in the thighs and breasts. Physical examination revealed an acutely ill white female in deep coma. The deep reflexes and blood pressure were unobtainable. She died 1 hour after admission. Autopsy revealed a massive recent hemorrhage in the right lobe of the cerebellum occupying four-fifths of the white matter with extension to the vermis and the regional cortex (Fig. 1). The ventricles were filled with fluid blood. In addition, there were small focal hemorrhages in the corpus callosum and midbrain. Examination of the viscera revealed extensive leukemic infiltration of the bone marrow, spleen, liver, left breast, subcutaneous tissue of both thighs and antecubital fossae, trachea, heart, stomach (with massive ulceration), renal medullary pyramids and renal cortex. Microscopy confirmed extensive leukemic infiltration in the sites mentioned and in the region of the cerebellar hemorrhage (Figs. 2 and 3). The final diagnosis was an acute exacerbation of a chronic myelogenous leukemia with fatal terminal cerebellar hemorrhage.

Case 2

J. S. (A-39-256), a white female, 5 years old, entered the hospital because of weakness, loss of weight and purpura. Hematological studies established the presence of a chronic myelogenous leukemia with an acute exacerbation. Respiratory distress was noted terminally after a prolonged downhill course. In addition to widespread leukemic infiltration of all lymph node structures and most of the viscera, an area of recent hemorrhage, 4 by 2½ cm., was found in the left cerebellar hemisphere in the region of the dentate nucleus near the vermis (Fig. 4). The

* Received for publication, January 22, 1942.

Case 9

D. L. (X-40-95), a white male, 58 years old, was found dead. Examination of the brain showed excavation of one-half of the cerebellum by a large recent hemorrhage (Fig. 6). Blood-clot casts of the fourth and other ventricles, and limited subarachnoid hemorrhage, were present.

Case 10

M. deA. (X-41-117), a white female, 76 years old, died suddenly at home. At autopsy, the left lobe of the cerebellum was replaced by reddish blood clot with extension to the subarachnoid space.

Case 11

A white female, about 65 years of age, of unknown identity (X-39-137), was found dead in the street. Autopsy revealed an area of hemorrhage, 4 by 5 cm., involving the right dentate nucleus and regional white matter. The medial portion of the left lobe was also affected by the hemorrhage. Extension to the subarachnoid space was found.

Case 12

M. F. (X-41-270), a white female, 21 years old, in the sixth month of pregnancy, complained suddenly of severe dizziness while shopping. She had had no previous complaints referable to her pregnancy. Within a very short time after the onset of dizziness, she became comatose and died before medical attention reached her. At autopsy, a large recent hemorrhage, 8 by 6 cm., involved the vermis with limited extension to both adjacent hemispheres. Both dentate nuclei were preserved. Only moderate subarachnoid hemorrhage was noted locally. On section, a complete blood-clot cast of all ventricles was found. The heart weighed only 200 gm. No aneurysm or other unusual findings were found after a careful search.

Case 13

S. K. (A-38-99), a white female, 52 years old, known to have had hypertensive heart disease for many years, entered the hospital with diminished cardiac reserve and headache. The systolic blood pressure was 260 mm. and the diastolic pressure, 140 mm. The blood urea nitrogen was 56 mg. per cent. Because of drowsiness, uremic odor of the breath, papilledema, albuminuria and enlarged heart, a diagnosis of malignant nephrosclerosis was made. Autopsy confirmed the clinical impression. In addition, a small hemorrhage (2 cm. in diameter) was found in the left lobe of the cerebellum situated in the deep cortical layers near the junction of gray and white matter.

Case 14

J. K. (A-40-505), a white male, 52 years old, with a history of hypertension of 3 years' duration, entered with the complaint of inability to void. No obstructive genitourinary lesion was found to account for the retention. The blood pressure ranged from 200 to 280 mm., systolic, and 100 to 180 mm., diastolic. The blood urea nitrogen rose from 59 to 120 mg. per cent during 22 days in the hospital. No neurological symptoms or signs were noted. The immediate cause of death was an extensive bronchopneumonia of the left lower lobe. The kidneys showed advanced arteriolosclerosis, without necrotizing changes. The left ventricular myocardium was massively hypertrophied, the heart weighing 710 gm. The brain showed no unusual features, with the exception of the right cerebellar hemisphere, where an area of dark hemorrhage, 2 by 1 cm., was seen involving the cortex, with limited regional extension to the subcortical white matter (Fig. 7). The ventricles and subarachnoid space contained no blood.

hemorrhage had extended through the ependyma of the fourth ventricle with a complete blood clot cast of all the ventricles. Microscopically, large numbers of immature myeloid cells were seen intimately associated with the hemorrhage.

Case 3

E. R. (A-40-154M), a white male, 74 years old, suddenly complained of a violent headache. He became comatose within a short interval and died several minutes after admission to the hospital. Autopsy revealed a massive hemorrhage almost completely filling the right cerebellar hemisphere, with extension to the vermis, and subependymal hemorrhages beneath the fourth ventricle. The ventricular cavities contained no blood. Regional subarachnoid hemorrhage of moderate degree was present. Secondary streaked hemorrhages were seen in the pons. The heart was moderately hypertrophied, weighing 500 gm.

Case 4

M. W. (A-39-84), a white female, 65 years old, suddenly became dizzy and nauseated while riding on the subway. On admission to the hospital, she complained of severe headache. Definite nystagmus was present with the slow component to the right. The systolic blood pressure was over 300 mm. Spinal tap yielded bloody fluid, under markedly increased pressure. She expired after 6 hours. The immediate cause of death was a massive recent hemorrhage completely destroying the dentate nucleus and regional white matter of the left lobe of the cerebellum (Fig. 5). All ventricles were filled with blood clot and fluid blood. In addition, an old area of softening was found in the right basal ganglia, with limited hemorrhage. Marked cerebral atherosclerosis, arteriolar nephrosclerosis and hypertrophy of the left ventricle were also noted.

Case 5

S. S. (A-39-227M), an elderly white male, died suddenly with no previous history available. Autopsy findings consisted of massive cerebellar hemorrhage, arteriolar nephrosclerosis, moderate hypertrophy of the heart (420 gm.) and marked cerebral atherosclerosis. In the right cerebellar hemisphere there was noted a large cavity 12 cm. in diameter, completely filled with dark red clotted blood. There was limited extension to the left lobe. All ventricles were occupied by dark red, fluid and clotted blood. The cisterna-magna contained approximately 4 cc. of blood.

Case 6

C. K. (X-39-202), a white male, 63 years old, had known that he suffered from heart disease and diabetes for 2 years. On the morning of death, he noted the sudden onset of "terrific" headache and dizziness. He died within 1 hour, before medical examination was available. At autopsy, practically the entire right cerebellar hemisphere was replaced by a large hemorrhage which extended to the subependymal region of the fourth ventricle. None of the ventricles contained blood. The heart weighed 720 gm.

Case 7

E. H. (X-39-162), a colored female, 35 years old, had been treated for hypertension for at least 1 year. She was found dead by her daughter. Autopsy revealed massive hemorrhage into the right cerebellar hemisphere, with extension to all ventricles and basal cisternae.

Case 8

A white male, 65(?) years old, of unknown identity (X-39-178), was found dead. The cause of death was a large hemorrhage in the vermis of the cerebellum with more marked extension to the right lobe and a blood-clot cast of all ventricles.

did not differ from those noted in other forms of intracerebellar disease except for the dramatic suddenness of onset. In the 10 instances in which pain in the back of the neck was noted, a very typical history was that of a sensation of being "struck a blow on the back of the neck."

In 6 of the cases in series B, death was sudden, so that no definite history was available. No symptoms referable to the central nervous system were described in 4 other instances. In 3 of these, the hemorrhage was of such limited extent that the absence of symptoms is not surprising. It is of interest to note at this point that all 3 occurred in cases of uremia. In the fourth, although the hemorrhage ruptured into the fourth ventricle, the symptoms were masked by the advanced general debility of leukemia. Dizziness occurred in 4 of the remaining 5 cases and was characterized again by the dramatic suddenness of onset. Severe headache was noted in 1 case, while rapidly progressive coma, soon after the onset of symptoms, was observed in 2 instances.

Neurological Signs. There was no constant pattern of signs elicited in these cases of cerebellar hemorrhage. Of the 109 cases reviewed from the literature, by far the majority exhibited no neurological signs other than coma. In 18, hemiparesis or hemiplegia was noted and in two-thirds of these was on the side opposite the hemorrhage. Cranial nerve palsies were observed in 12 instances, the facial nerves in 6, the ocular nerves in 5, while the hypoglossal nerve was involved in the other. The facial palsy was homolateral in 4 of the 5 cases in which the site of the hemorrhage was stated. Conjugate deviation of the eyes was observed in 7 instances and was contralateral in 4 of the 5 cases in which the location of the lesion was described. Other less frequent and variable neurological signs included nystagmus (5), adiadokokinesis (2), opisthotonos (3) and positive Romberg's sign (2).

In series B, no significance can be attached to the relative incidence of symptoms and signs, inasmuch as 8 individuals died suddenly or before medical attention was available, and 3 represent incidental minimal lesions. One case showed marked nystagmus. Another revealed absence of the deep reflexes and unilateral flaccidity. In a third, admitted in coma, the deep reflexes were absent. In 13 cases, no neurological signs were present.

Duration. The duration of life from time of onset of symptoms until death may be seen from a study of Table II. That in series A there were only 3 examples of either sudden death or "found dead," while in the much smaller series B there were 6 such instances may be accounted for by the fact that many of the autopsies in the latter series were upon "sudden deaths" investigated by the Medical Examiner's Office. In

Case 15

W. L. (A-41-284), a white male, 48 years old, entered with progressive urinary complaints, and died in uremia after 8 days, with signs of ascending urinary infection. No neurological symptoms or signs were noted during the course of his illness. The essential mechanism of death was an extensive cystitis and ureteropyelonephritis secondary to prostatic hypertrophy. A small hemorrhage, 2 mm. in diameter, was found in the right cerebellar hemisphere at the junction of the gray and white matter (Fig. 8). There was no extension to the subarachnoid space or ventricles.

Clinical Data

The two series will be compared in the following respects: age, sex, predominant symptoms, outstanding neurological signs and duration. The central nervous system lesions and the associated or related systemic pathology will be considered separately.

Age. In series A there were 8 cases in which the age was not stated and in series B, 2 such instances. The distribution of the remaining cases is presented in Table I.

Table I

Age Distribution by Decades of 114 Cases of Cerebellar Hemorrhage

Decade	Series A	Series B
1	4	1
2	11	0
3	13	1
4	6	1
5	10	1
6	14	4
7	23	3
8	18	2
9	2	0
	101	13

Sex. In series A, there were 61 males, 46 females and 2 cases in which the sex was not stated. In series B, 7 were males, and 8 females.

Symptoms. A complete and detailed account of the symptomatology of cerebellar hemorrhage may be culled from an analysis of the literature. Coma as a prominent symptom far overshadowed all other findings, having been recorded in 69 cases. In the order of frequency, the following additional symptoms were noted: vomiting (38), severe headaches (35), dizziness (16), ataxia (11), pain in the back of the neck (10), convulsions (5), recurrent mania (3), syncope (3), and blindness, abdominal pain and respiratory difficulty in 1 instance each.

The coma was usually of a profound type and as a rule premonitory aura were absent. Vomiting as the only symptom was rarely present and on a few occasions was described as projectile in nature. The distribution of the headache was not constant, but tended to be occipital rather than frontal in location. The dizziness and ataxia, when present,

sclerosis of moderate to marked degree was noted in 3 of the 15 cases reported in series B.

Miscellaneous Cerebral Lesions. In series A, 1 instance each of gumma of the cerebellar vessels with rupture and hemorrhage, and of ruptured miliary aneurysms was noted.

Hypertrophy of the Heart. Cardiac enlargement was described in 11 cases in series A. As far as could be determined, no heart weights were recorded in these cases. In series B, 10 cases of hypertrophy of the heart were observed. In 1, the weight was not recorded, while in the others weight of the hearts varied from 420 to 720 gm., with an average of 565 gm.

Other Incidental Findings. In series A, 1 case each of hydatid cysts of the liver, healed rheumatic heart disease, cirrhosis of the liver and ulcerative colitis was mentioned. In series B, 3 cases of malignant nephrosclerosis and 2 cases of myelogenous leukemia were seen; 3 patients died in uremia. None of these 3 had focal symptoms of the central nervous system, and in all there were found only small hemorrhages, 2 mm., 2 cm., and 2 by 1 cm. in diameter, respectively. One patient expired during the sixth month of gestation and no associated pathological changes were found in any of the viscera. The heart in this instance weighed only 200 gm., and there was no demonstrable alteration of the cerebral vessels.

Comment

Determinations of the relative frequency of cerebellar as compared to cerebral hemorrhage have varied from 0.9 to 10 per cent.[2] In our own series, the ratio of cerebellar to cerebral hemorrhage was 15 per cent. As indicated in the discussion of the duration of symptoms, this higher incidence is explained by the inclusion of Medical Examiner's material. Of the 94 cases of spontaneous intracranial hemorrhage observed in the hospital, only 8, or 8.5 per cent, were of the cerebellar variety, whereas of the 21 instances of spontaneous intracranial hemorrhage that died outside of the hospital 7, or 33 per cent, were located in the cerebellum. Because this occurrence of cerebellar hemorrhage did not seem to be disproportionate to the relative mass of the cerebellum, the weights of cerebellum and cerebrum were recorded in 50 consecutive brains. The ratio between the average weights was 12.3 per cent. If no specific or peculiar factors were operative in the causation of cerebellar hemorrhage, one would expect this figure to represent the ratio of incidence of cerebellar to cerebral hemorrhage. Actually, the incidence in our series exceeds this figure. If the 3 cases of incidental minimal hemorrhage are excluded, it is of interest to note that the ratio

series A, 48 per cent of the patients died after 24 hours. None of those in series B lived for more than 9 hours.

Pathology in the Cerebellum

Location. In series A, hemorrhage occurred in the left lobe in 43 cases, in the right in 49 and in the vermis in 15. The corresponding figures for series B are 4, 8 and 3.

Table II
Duration of Life from Time of Onset of Symptoms until Death

	Series A		Series B	
Sudden death	3		6	
Less than 1 hour	9		3	
One to 12 hours	27		2	
Twelve to 24 hours	14		..	
Total (less than 24 hours)		53		11
One to 7 days	23		..	
One to 4 weeks	16		..	
One to 6 months	8		..	
More than 6 months	2		..	
Total (more than 24 hours)		49		0
Grand total		102		11

Size. The size of the hemorrhage was noted specifically in 104 of the 109 cases reviewed. In 84, the hemorrhage exceeded 5 cm. in diameter. In series B, 11 were larger and 4 smaller than 5 cm. Of the latter 4, 3 were incidental lesions, without clinical significance.

Extension of Hemorrhage. There were 7 examples of extension to the subarachnoid space in series A, and 4 in series B. Ventricular extension of the hemorrhage was more frequent and included 35 instances of this complication in series A. In 30 of these, the hemorrhage in the cerebellum was listed as larger than 5 cm. Of the 35 cases of intraventricular extension, 26 lived for less than 24 hours. In most of the cases cited in the literature, the findings in the ventricles were not mentioned specifically. Of the 8 cases of intraventricular extension in series B, 5 were found dead or died suddenly, 1 lived for 1 hour and 2 lived for 9 hours. Hemorrhage into the ventricles was definitely noted in 4 of the 6 cases of sudden death, while the condition of the ventricles was not specifically stated in the other 2 cases.

Associated Pathology

Cerebral Arteriosclerosis. Cerebral arteriosclerosis was mentioned in the protocols of 14 of the cases cited in series A. A quantitative estimation of the degree of sclerosis was not stressed. Cerebral arterio-

our own experience with these three cases of incidental hemorrhages, it is suggested that careful examination of the cerebellum be carried out in those dying of uremia.

Conclusions

1. Cerebellar hemorrhage is similar to cerebral hemorrhage in incidence and the contributory pathological factors are identical.

2. Cerebellar hemorrhage tends to be more rapidly fatal than cerebral hemorrhage and produces a more variable clinical syndrome.

Note: We wish to thank Dr. Richard Grimes, Assistant Medical Examiner in Queens County, for permission to include the Medical Examiner's material.

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is reduced to 12 per cent, exactly the ratio of the weight of the cerebellum to the weight of the cerebrum. These findings contradict the general impression that cerebellar hemorrhage is a rare lesion, in contrast to cerebral hemorrhage. The alleged rarity previously stressed is explained in part by the failure to investigate adequately cases of sudden death, which constitute a considerable proportion of the present series.

Cerebellar hemorrhage tends to be fatal more rapidly than cerebral hemorrhage for several reasons. The proximity of vital medullary centers, the confining effect of the tentorium cerebelli and the relatively short tissue barrier from the subarachnoidal space and the ventricular system are all important factors. Extension of the hemorrhage into the ventricular system is a cause of sudden death in all forms of intracranial hemorrhage, and this complication occurs more often in cerebellar apoplexy. Of the 12 cases in which the cerebellar hemorrhage was the cause of death in our series, 8 showed intraventricular extension.

On the basis of simple anatomical and physiological grounds, there is no reason to expect that the factors responsible for cerebellar hemorrhage should differ in any major degree from those causing cerebral apoplexy. Actually, this is borne out by an analysis of associated pathological findings and case histories. Hypertensive cardiovascular disease was the most common associated finding. In only 2 of the cases was a heart of normal size recorded. This parallels the usual findings in cases of cerebral hemorrhage. Malignant nephrosclerosis, cerebral arteriosclerosis and myelogenous leukemia, common findings in association with cerebral hemorrhage, were noted in 8 instances. The identical nature of the contributory pathological factors in cerebellar and cerebral apoplexy has not been stressed in the literature heretofore.

The clinical syndrome of cerebellar hemorrhage is a variable one, in contrast to the typical example of cerebral hemorrhage. When the hemorrhage is small, the lesion is often silent, as might be expected. When the lesion is large, it is usually rapidly fatal. No characteristic syndrome, corresponding to that found in hemorrhage in the internal capsule and basal ganglia, is observed in cerebellar hemorrhage. The common site of cerebral hemorrhage is the internal capsule and regional tissue, a central location in the cerebrum. A similar central localization of cerebellar hemorrhage in the region of the dentate nucleus or vermis has been observed. In the three small incidental hemorrhages described in series B, however, these structures were not affected. In contrast, the small lesions were noted at the junction of the white matter and the cortex, usually at the periphery of the affected lobe. On the basis of

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Spontaneous Cerebellar Hemorrhage

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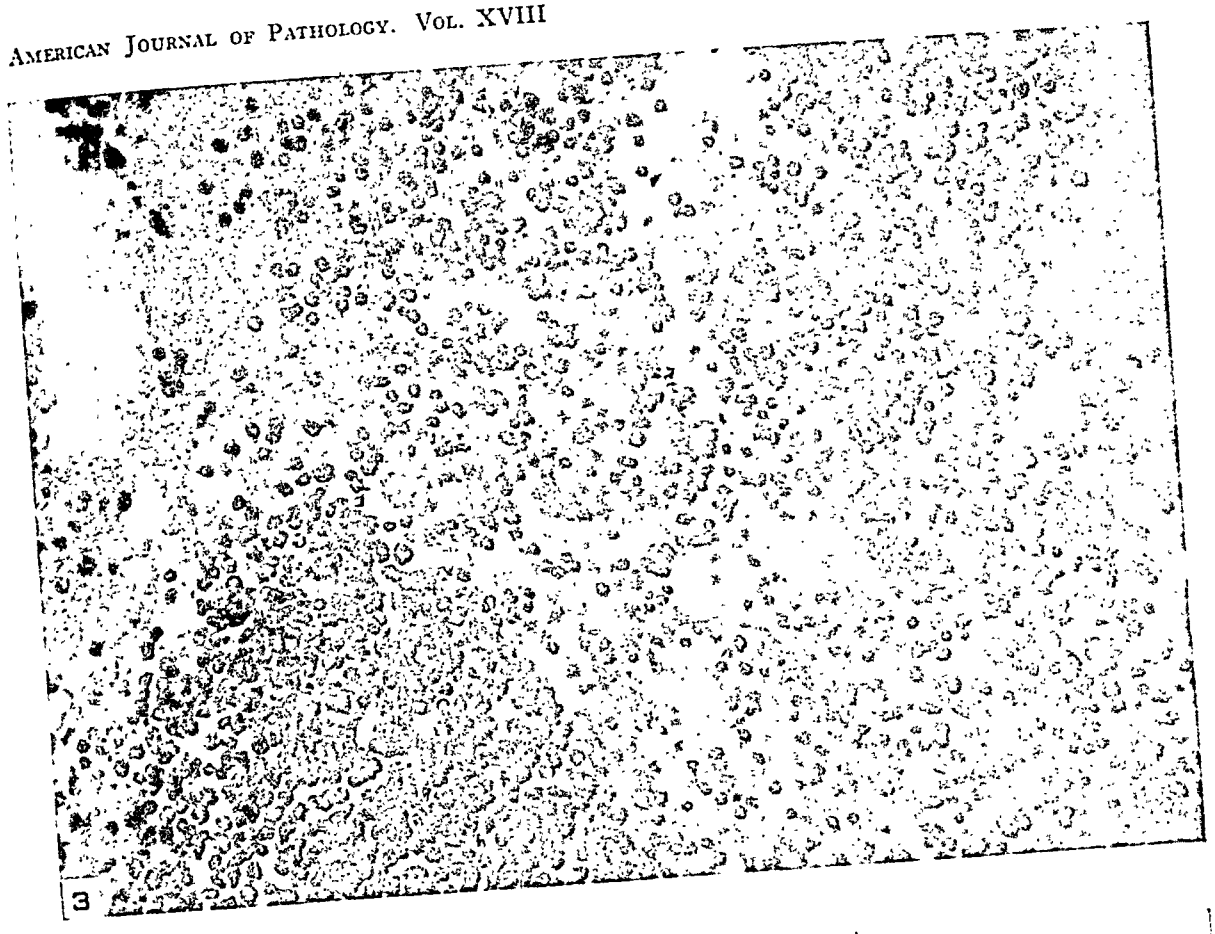
DESCRIPTION OF PLATES

PLATE 142

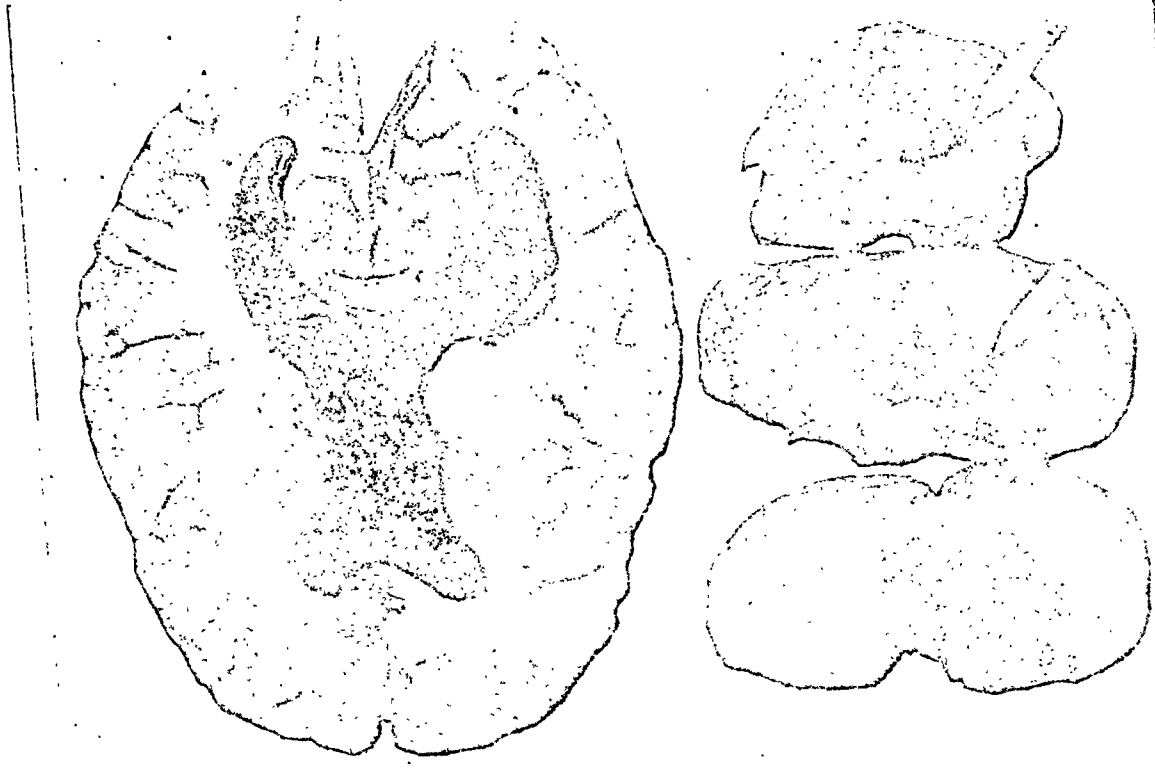
FIG. 1. Case 1. Massive hemorrhage of right lobe, extending to the vermis.

FIG. 2. Case 1. Low power photomicrograph of cerebellum showing leukemic infiltration and hemorrhage in white matter adjacent to molecular layer of cortex. × 80.

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PLATE 143

FIG. 3. Case 1. High power photomicrograph of cerebellum showing leukemic infiltration at lower left and hemorrhage at upper left in white matter. × 205.

FIG. 4. Case 2. Hemorrhage into left lobe of cerebellum in region of dentate nucleus with extension to fourth and lateral ventricles.

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Spontaneous Cerebellar Hemorrhage

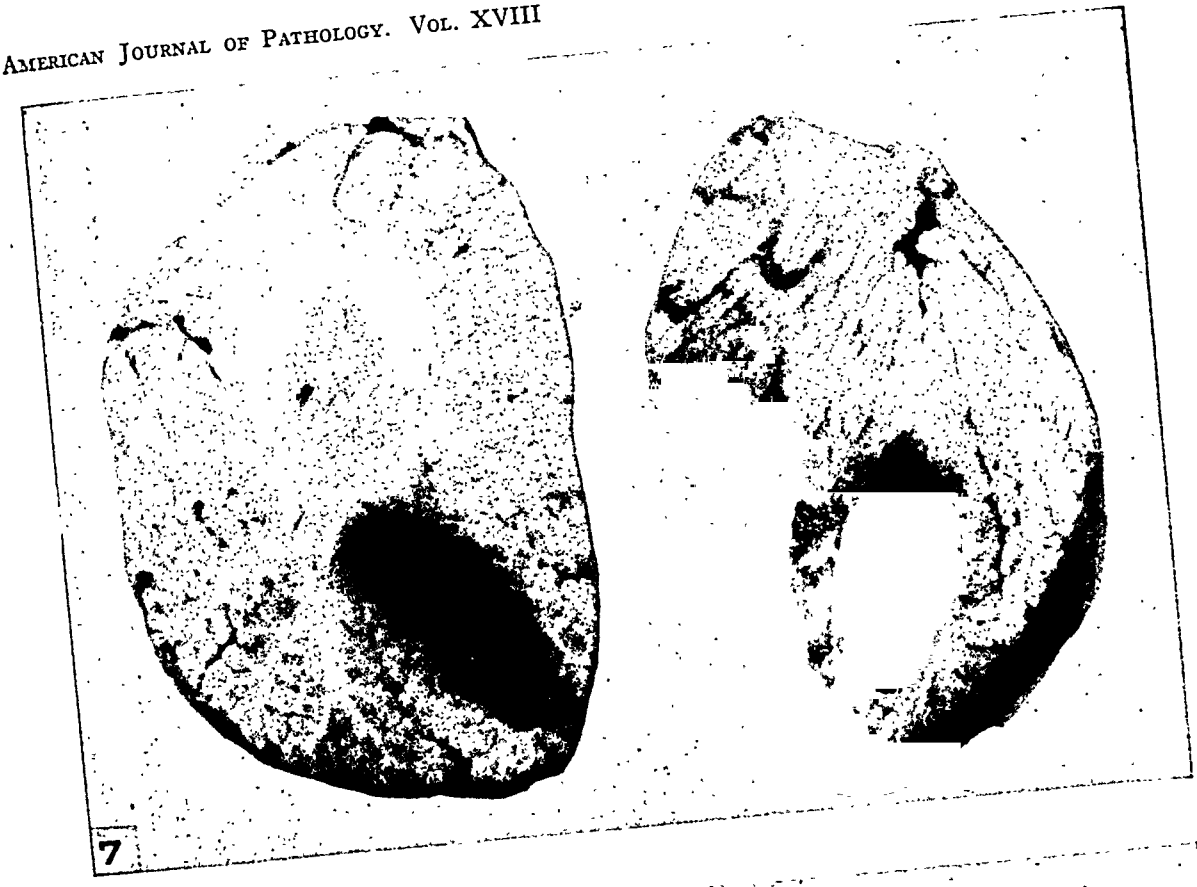
Mitchell and Angrist

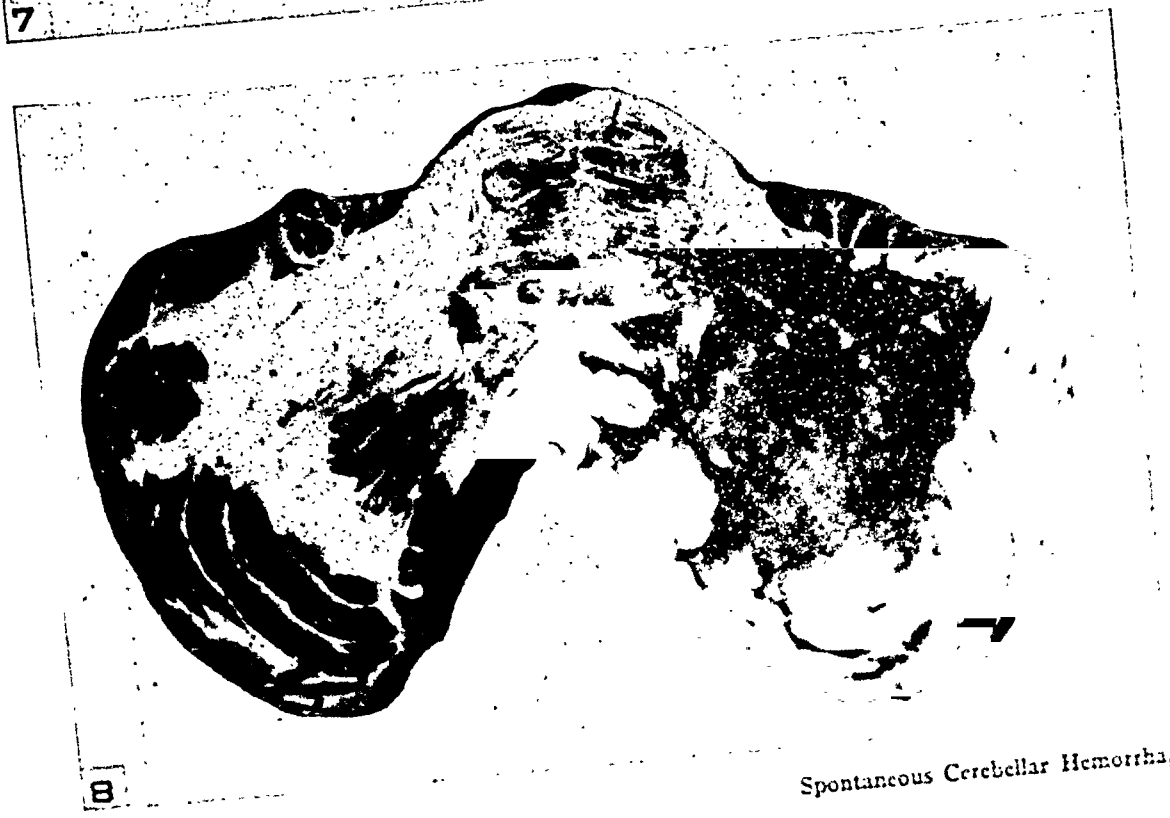
PLATE 144

FIG. 5. Case 4. Sagittal section of cerebellum, pons and upper medulla showing large hemorrhage in vermis extending to fourth ventricle.

FIG. 6. Case 9. Centrally placed massive hemorrhage in left lobe and vermis with extension to lateral ventricle.

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Spontaneous Cerebellar Hemorrhage

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PLATE 145

FIG. 7. Case 14. Right lobe of cerebellum showing hemorrhage, 2 by 1 cm., in cortex and adjacent white matter.

FIG. 8. Case 15. Horizontal hemisection of cerebellum and pons through the region of the middle cerebellar peduncle, with an area of hemorrhage, 2 mm. in diameter, in right lobe at junction of gray and white matter.

ANLAGEN AND REST TUMORS OF THE LUNG INCLUSIVE OF "MIXED TUMORS" (WOMACK AND GRAHAM) *

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It is quite apparent from perusal of the literature relative to the classification of tumors of the lung that there is but little agreement upon this subject. The greatest attention is, of course, focused upon the study of tumors of bronchiogenic origin because of their relative importance and comparative frequency. The possibility of treatment by surgical methods and the intrinsic malignant or benign character of the neoplasm are factors of vital consideration to the patient. For a comprehensive dissertation upon these phases of the subject the reader is referred to the paper by Goldman and Stephens [1] with the accompanying discussions.

Aside from the representative and basic studies of Fried [2-4] upon bronchiogenic carcinomata, wherein aberrant growth arising from the basal cell of mature tissue structure is discussed, the most significant observation relating to the pleomorphism of pulmonary neoplasms in respect to both cytology and histological pattern is that of Womack and Graham [5] upon mixed tumors. We [6] have, in a previous communication, discussed the intricate embryological mechanism of the lung and the likelihood of anlagen and rest alterations as a source of neoplastic formation. The intimacy of the laryngotracheal ridge and esophageal development serves to explain the entodermal anomalies of epithelial structures as emphasized by Rector and Connerley.[7] The mesenchymal defects are exemplified in congenital cystic disease, which topic, in general, is well set forth by Sellors.[8] Conceding such formative digressions as one source of tumor origin, the possibility of bizarre and diverse histological manifestations becomes apparent. It is not unlikely that these factors have led to the confused deductions and to the various names objectively applied in efforts at classification.

The designation "mixed tumors" of the lung by Womack and Graham [5] has had the salutary effects of emphasizing the embryological course of certain pulmonary tumors and of carrying a reasonable implication that some of these are benign. Strictly speaking, many such tumors may be unidermal, but with the intention of both confirming and stressing the observations of Womack and Graham, it appears to us that the terms "anlagen" or "rest" tumors serve more fully to em-

* Received for publication, January 26, 1942.



CASE 2

This infant survived 5 days after full-term delivery. The upper lobe of the left lung presented two large, more or less rounded areas of ivory color which occupied the greater portion of its surface. Multiple radiating blood vessels, varying from approximately 1 mm. in diameter to minute dimensions, formed a cirsoid or telangiectatic arrangement over the surfaces (Fig. 3). Upon sectioning, these separate ivory-colored masses were found to merge beneath the surface. A structure of dense consistency was found to extend for a depth varying from approximately 1 mm. to 1 cm. It extended downward into a spongy reddish brown tissue which was less dense in character. The latter portion occupied approximately one-half of the upper lobe, the remaining tissue being crepitant and conforming to normal lung.

Microscopical Examination

In the pleural areas multiple blood-vascular sinuses were present within the connective tissue. These varied greatly in size and had thin walls. In many instances the lumen contained erythrocytes. Similar vessels appeared occasionally in the stromal projections within the underlying structure (Fig. 4). The nodules, as a whole, consisted of predominant young fibrous tissue. There were also numerous acinar spaces which were lined by low cuboidal epithelium and appeared in the aggregate as embryonal lung structure. Such, no doubt, had their origin from the entodermal layer of bronchial buds. A few irregularly shaped tubular spaces lined by columnar epithelium represented the more adult bronchiolar formations. It is probable that some of these latter spaces had certain continuity with the normally developed lung inasmuch as similar inflammatory cells were found scattered diffusely throughout spaces of both types.

CASE 3

A white male adult, 34 years of age, presented suggestive signs of an intrabronchial tumor, including hemoptysis. Specimens were obtained through bronchoscopic removal in 1935 and again in 1939. The interesting feature in this case is the dominance of one form of histological pattern in the primary biopsy specimen as compared to that removed 4 years later.

The specimen obtained in 1935 consisted of a small, rosette-shaped mass of tissue measuring approximately 6 by 5 by 4 mm. It was red, and soft. Upon removal, profuse bleeding occurred.

phasize and encompass their objective. Naturally, the benignancy or malignancy of such neoplasms are dependent upon the same histological criteria regardless of origin.

Material Studied

The first two tumors which suggested to us origin from anlagen or rests occurred in newborn, full-term infants who had survived 7 and 5 days respectively. Such distinctive intrauterine tumor growths immediately indicated embryological aberrations. The case histories and clinical findings are not important. There were evident respiratory difficulties and congenital atelectasis seemed probable.

CASE 1

In this infant surviving 7 days, it was found at necropsy that a tumor mass had replaced the entire lower lobe of the left lung. Upon sectioning, the mass was somewhat soft, and there were tubular structures, apparently dilated bronchi with thin walls, much like those noted in congenital cystic disease (Fig. 1). It is noteworthy that no irregular nodular formations were found except in that portion directed upward into the adjacent lobe. There had developed, for the greater part, a complete growth replacement of the normal structure of the lung. No other tumor masses and no metastasis were found.

Microscopical Examination

Sections prepared from various areas revealed, in general, the same histological picture. This consisted of irregularly distributed, tortuous branching tubules forming an apparently purposeless distribution of irregular alveolar spaces (Fig. 2). In many areas, infoldings or spur-like papillary invasions were noted. There was an occasional elongated tubular space having parallel sides and terminating in a simple bifurcation. These were suggestive of rudimentary bronchi. Tall, slender, columnar epithelial cells formed the linings for all the alveolar and tubular spaces. They revealed elongated, slender nuclei which were densely basophilic and presented a pale, slightly granular cytoplasm. In general these cells were arranged in a single layer, but occasionally they were stratified. The stromal structure was scant in amount and consisted of delicate, regularly arranged fibrous tissue. There was infrequently noted, in places where a larger amount of stroma was grouped, a more or less oval plaque of embryonal cartilage. Such plaques bore no direct relationship to the pseudobronchi and apparently were simple outgrowths of the mesenchymal development. Here and there in the stroma were found nutrient blood vessels.

tures indicative of cartilage formation were seen. As has often been noted in growths in such locations, marked squamous-cell formation, metaplastic or aberrant, was present over the surface. The general characteristics of this tumor were indicative of mixed germinal origin inasmuch as both entodermal and mesenchymal structures appeared as participants in the neoplasm.

Summary

Four tumors of the lung, manifesting distinct evidences of origin either in anlagen or from more than one germinal layer, have been described. Two of these were of definite embryonal or anlagen origin, having developed during intrauterine life. In the first instance embryonal bronchial budding was perpetuated in a neoplastic mass. This was demonstrated by an aimless reduplication of tubular and immature alveolar structures indicative of a lack of orderly sequence of bud-branching. In the second case, evidence of both entodermal and mesodermal origin was revealed by areas of varied predominance of one or the other derivatives. In the third instance, less evidence of origin from mixed dermal layers was observed in each of two tissues removed for biopsy. Comparison, however, of the two specimens, between which there was an interval of 4 years, revealed a definite emphasis upon mesodermal structure in the first, while an entodermal character was clearly shown in the latter specimen. It is our opinion that the neoplasm in this second biopsy conformed closely in histological aspect to embryonal glands. The fourth example presented a neoplasm of mixed dermal components as revealed by the prevalence of elements of both layers.

It seems imperative, from the standpoint of the histological study of bronchiogenic tumors, that the embryological nature, as emphasized by Womack and Graham,[5] must receive serious consideration. In the study herein reported, bronchial anlagen origin is clearly established for certain of the tumors presented. Origin in an embryonal glandular rest is most probable in one instance.

While the biological characteristics of malignancy and benignancy are more or less constant regardless of the primary mature or embryonal origin of neoplasms, there are precedents for a benign nature for many tumors having the latter origin. Such serve to promote conservatism in the evaluation of the relative malignancy of the pulmonary growths which may belong to this group.

Note: We wish to express our appreciation to Dr. S. H. Colvin, through whose courtesy we obtained cases 3 and 4.

Microscopical Examinations

The sections presented a predominant mesenchymal derivative of cavernous hemangiomatous nature. There were numerous sinuses of irregular shape and size, lined with small rounded cells and containing numerous erythrocytes (Fig. 5). There were also similar spaces filled with cells resembling those present in embryonal bronchial glands.

The specimen removed in 1939 was an elongated oval portion of tissue of soft consistency and measuring 2 by 3 mm. in its greater diameters. Numerous more or less rounded acini were present. Many of these were lined with epithelium while others were completely filled with such cells. These cells presented comparatively large, deeply stained nuclei and only a small amount of light-staining protoplasm. They conformed to an embryonal or fetal gland type and were definitely of entodermal origin (Fig. 6). Only occasionally a sinus was seen containing blood, such as had been noted in the first specimen.

It seems evident that these two specimens were representative of mixed germinal layers. In the first a mesenchymal derivative was predominant while in the latter the tumor growth consisted to a great extent of elements of entodermal origin. This patient has just reported for observation. He is in excellent health and is free from all former signs and symptoms.

CASE 4

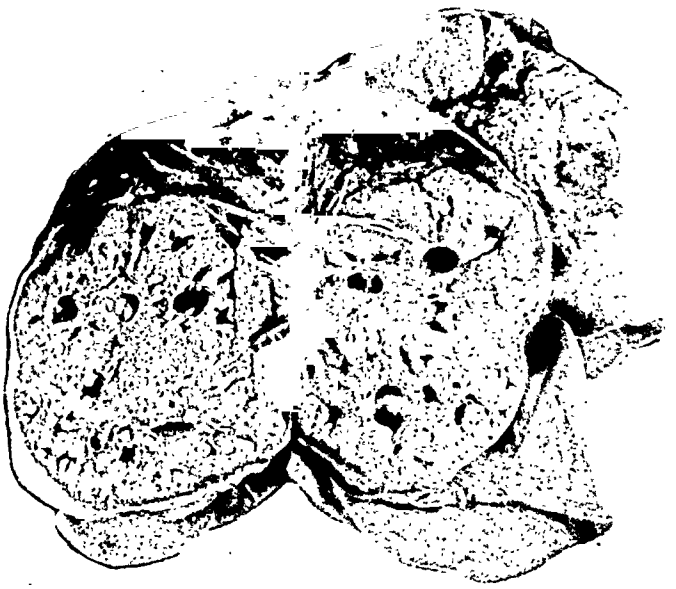
This individual was 19 years of age and had a history of occasional hemoptysis since the age of 7 years. Other clinical signs and symptoms were those usually present in a persistent intrabronchial polypoid growth wherein ball-valve action is present.

The specimen obtained through the bronchoscope was an irregularly shaped portion of tissue, of light pinkish red to pinkish gray color and somewhat firm in consistency. It measured approximately 7 by 5 mm.

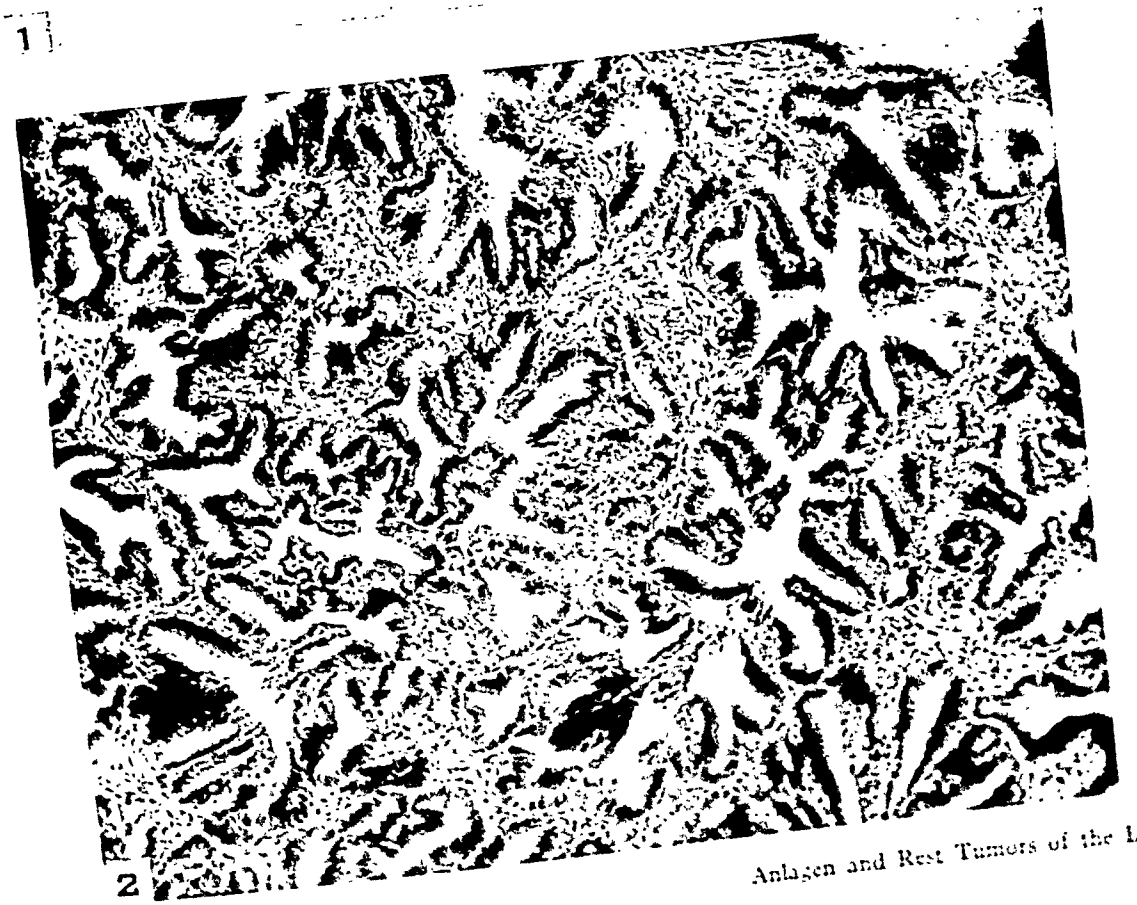
Microscopical Examination

The sections revealed many more or less rounded acini with a lining of somewhat "ragged" epithelial cells with pale-staining nuclei and cytoplasm. These acini suggested a somewhat mature mucous gland structure; yet they were not of the fully developed adult type. In many of these spaces were found basophilic concretions of irregular shape, some of which resembled corpora amylacea (Fig. 7). While in portions the stromal structure showed nothing noteworthy, in other areas it formed very extensive plaques and presented extensive hyalinization (Fig. 8). In some of these hyalinized areas, cellular struc-

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Anlagen and Rest Tumors of the Lung

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DESCRIPTION OF PLATES

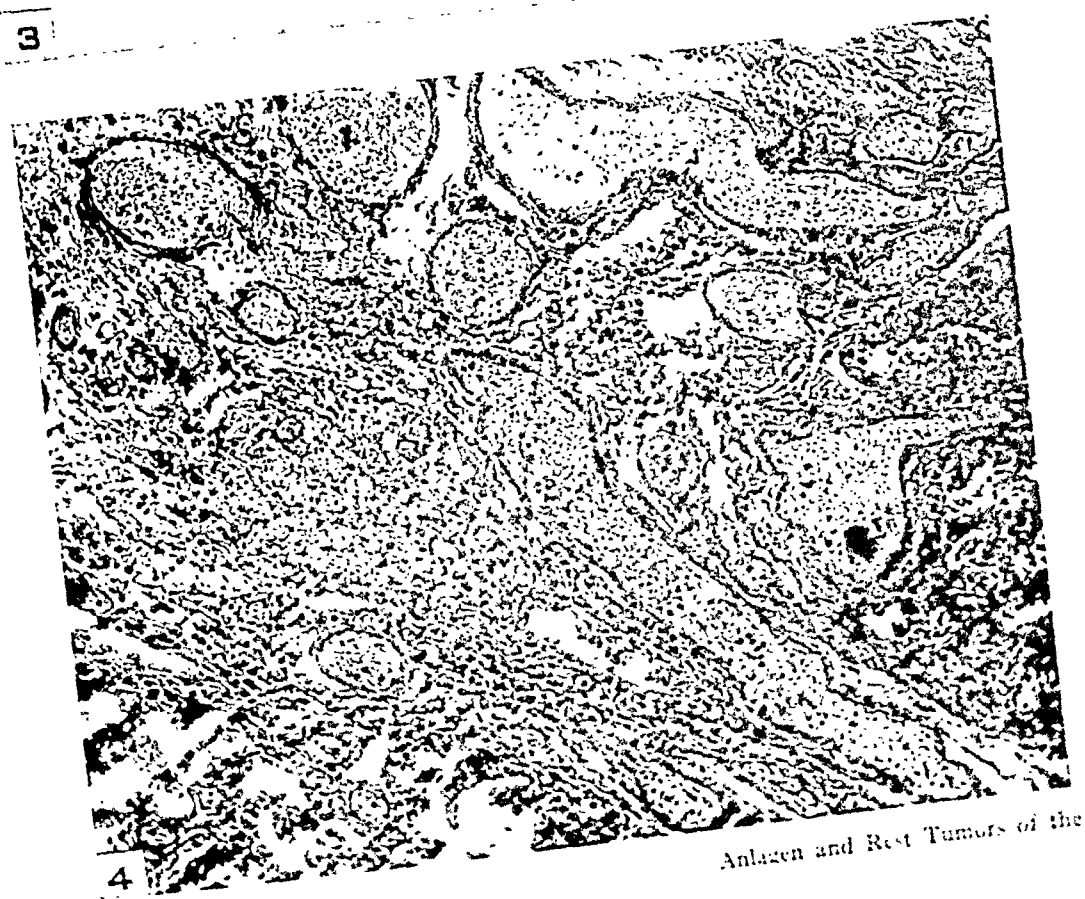
PLATE 146

FIG. 1. Gross photograph of the left lung in case 1 showing a tumor mass replacing the entire lower lobe and compressing the upper lobe. The openings represent dilated tubules of apparently undeveloped bronchi analogous to those which occur in congenital cystic disease.

FIG. 2. Photomicrograph of the lung tumor in case 1 revealing branching tubules similar to those present in early embryonal lung structure. The lining consists of a single layer of columnar epithelium. × 300.



3



4

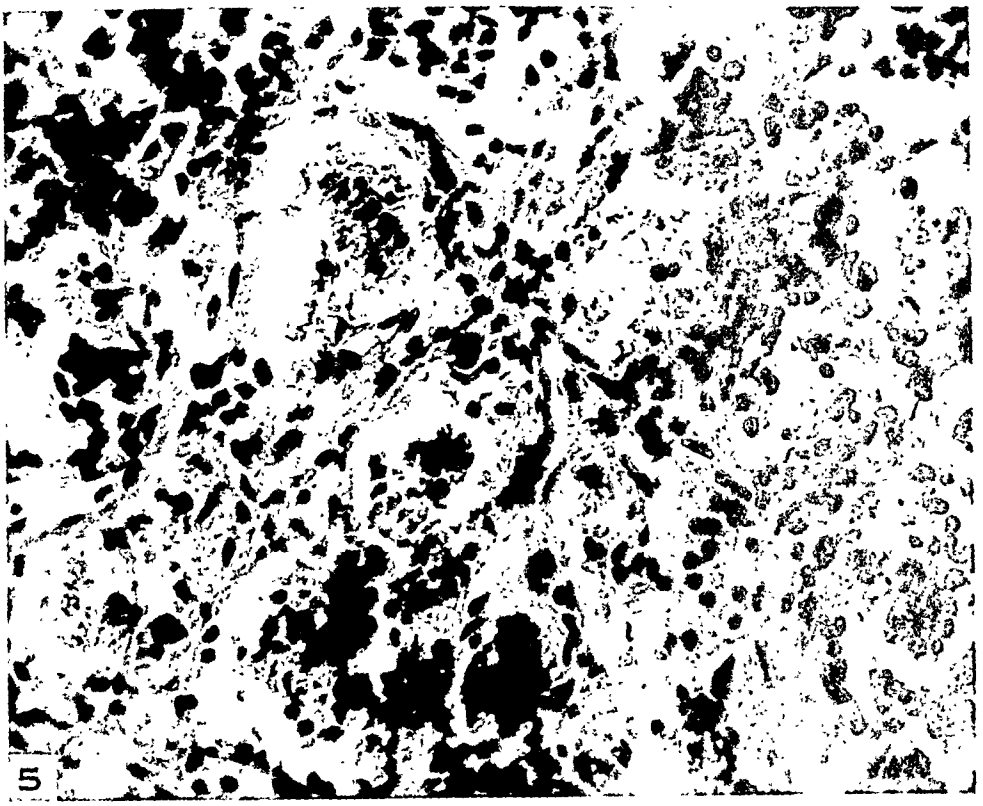
Anlagen and Rest Tumors of the Lung

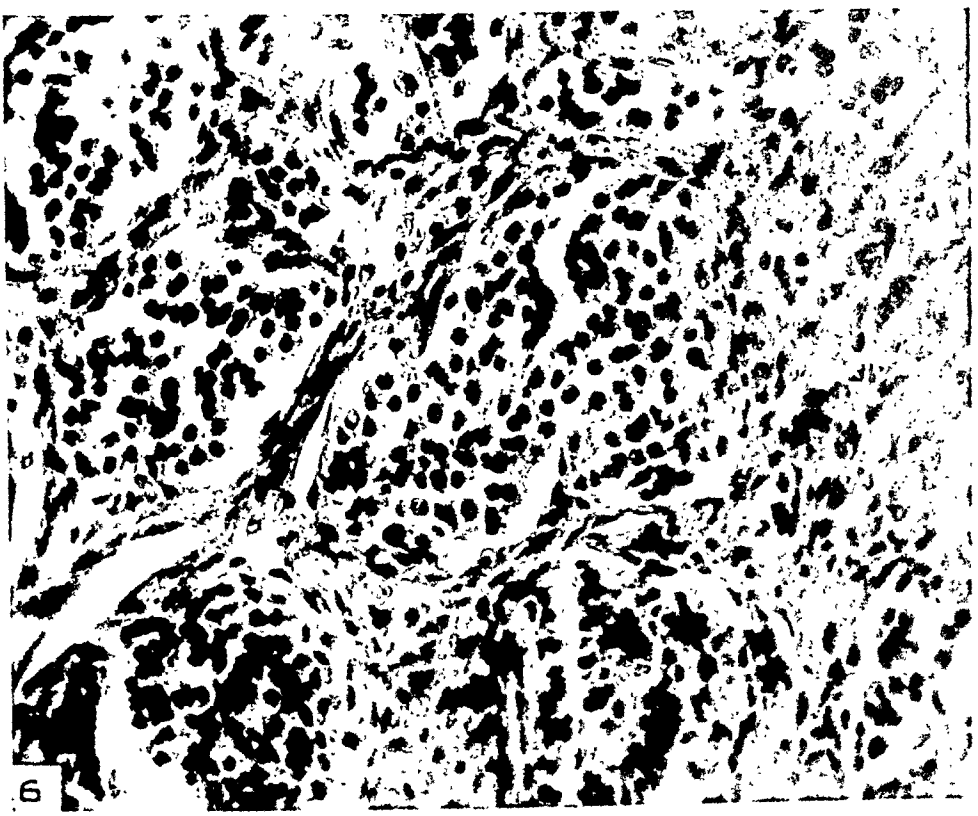
Harris and Schattenberg

PLATE 147

FIG. 3. Gross photograph of the right lung of case 2. Large tumor masses are seen in the upper lobe of the right lung. Dilated vascular ramifications are present.

FIG. 4. Photomicrograph of a section of the tumor in case 2 wherein cavernous sinus formation and rudimentary alveolar arrangements of lung structure can be noted. × 200.





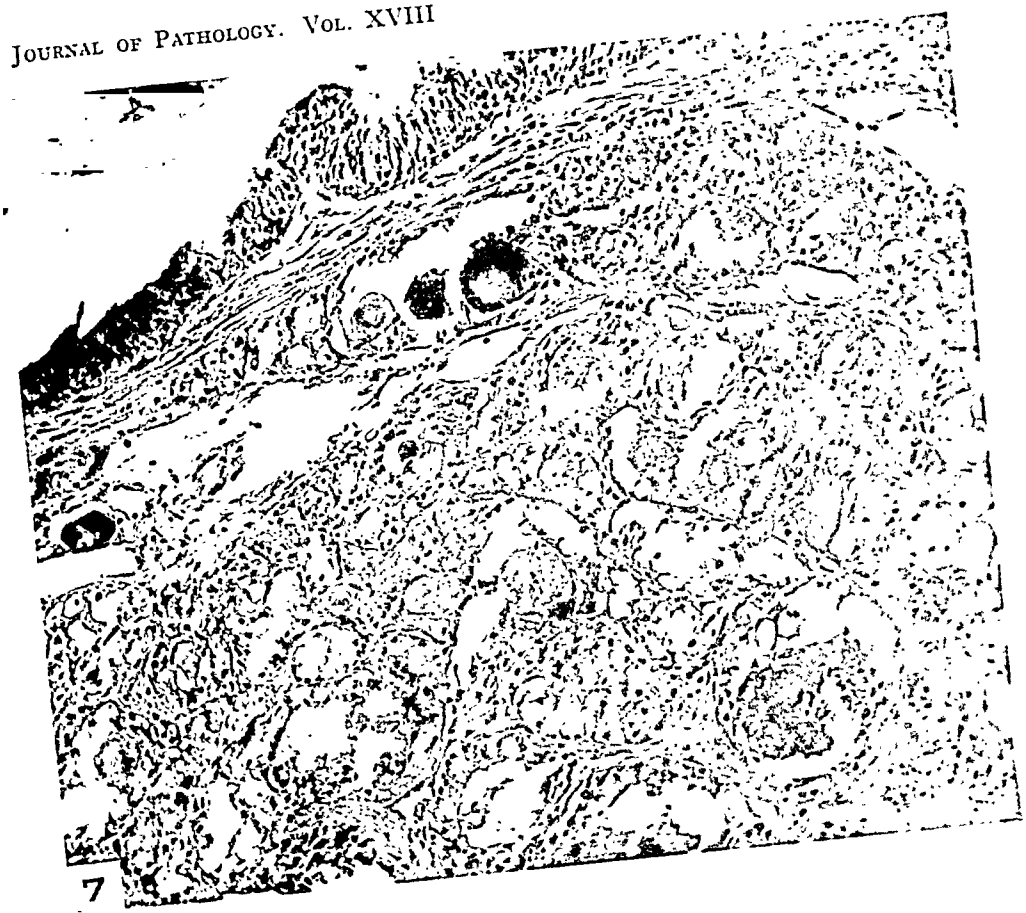
Harris and Schattenberg Anlagen and Rest Tumors of the Lung

PLATE 148

FIG. 5. Photomicrograph of the biopsy specimen in case 3, obtained in 1935, in which angiomatous formation is predominant. Numerous vascular spaces containing erythrocytes are shown. × 500.

FIG. 6. Photomicrograph of the biopsy specimen of case 3, removed 4 years later (1939) in which embryonal glandular structure prevails. Numerous acini contain round and polyhedral cells with deep-staining nuclei and pale protoplasm. × 500.

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PLATE 149

FIG. 7. Photomicrograph of the biopsy specimen of case 4, revealing glandular acini, many of which contain concretions. The lining cells are pale and irregular in outline. The columnar epithelium of the bronchial lining above shows squamous cell metaplasia in the upper left portion of the field. × 250.

3. Photomicrograph of the biopsy specimen of case 4, showing an area wherein ensive chondromatous structure prevailed. A few scattered glandular acini e present. × 250.

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THE AMERICAN JOURNAL OF PATHOLOGY

VOLUME XVIII NOVEMBER, 1942 NUMBER 6

BENIGN CHONDROBLASTOMA OF BONE *
A REINTERPRETATION OF THE SO-CALLED CALCIFYING OR CHONDROMATOUS GIANT CELL TUMOR

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Our comprehension of the lesion under discussion developed out of a study of the question of giant cell tumor of bone. That study [1] led us to a rather strict definition of what should be regarded as representing the latter lesion. Collaterally we were led to reject from the category of giant cell tumor, as independent entities, a number of other lesions usually interpreted as modifications or variants of this tumor. Among these rejected lesions is the one under consideration here, which has usually been described as the "benign calcifying" or the "epiphyseal chondromatous" giant cell tumor. We hold that this lesion is a specific type of tumor, developing from cells best interpretable as cartilage germ cells because of the aura of cartilage about the lesion as a whole, and we wish to designate the tumor as "benign chondroblastoma of bone."

This lesion presents certain special clinical features, but these are overshadowed in interest by its anatomic and particularly by its histologic peculiarities. The basic cells of the lesion are polyhedral cells of moderate size which may be closely compacted or somewhat separated by a small amount of interstitial ground substance. But what is striking and specific about the histologic picture is, in the first place, the presence of focal areas of calcification and the fact that where the cells and ground substance have become heavily encrusted with calcium the tissue is necrotic. Furthermore, in the necrotic areas one sees evidence of collagenization, resorption of the necrotic calcified débris and replacement of it by á hyaline chondroid tissue. The latter tissue itself may show patchy calcification and, in some places, even direct metaplasia into bone. This sketch of what we hold to be the distinctive pathologic features of the lesion omits various other aspects of the

* Received for publication, February 13, 1942.

lesion tends to be relatively small, that it is apparently seen only in young subjects and that it yields readily to conservative treatment (curettage with or without subsequent irradiation). Unlike Ewing [2,4] and Codman, however, we hold that, while starting its development in epiphyses and specifically in those of long bones, it does not exhibit predilection for the humerus. Furthermore, as already noted, we do not hold that the lesion, though distinctive, is to be regarded as a variant of giant cell tumor of bone, and believe instead that it is a peculiar tumor having a chondroblastic basis. Our discussion will be devoted mainly to an elaboration of this conception.

Clinical Features

Age and Sex Incidence and Localization

In all nine cases which we studied the patients were males. In Codman's [3] series of nine cases, six were males. The ages of our patients ranged within the narrow limits of 13 and 17 years and averaged 15.5 years. The ages of Codman's patients ranged between 12 and 24 years and averaged 17 years. These data indicate that it is in adolescent or postadolescent males that this lesion, apparently rather uncommon, is most likely to be encountered.

As to location, it has already been noted that in all of our nine cases it was a long tubular bone that was affected. The lesion was found limited to part of an epiphysis or involving the epiphysis and part of the adjacent metaphysis, but never located in a metaphysis exclusively. It may be surmised that the tumor originates in an epiphysis and that if the adjacent portion of a metaphysis is affected this involvement is secondary. In one of our cases, in which the patient was observed over a period of 16 months, during which several roentgenograms were taken, the growth of the lesion could be traced as it spread into the adjacent metaphysis.

In regard to our own cases, it was found that in four the lesion was in the lower epiphysis of a femur, involving also the adjacent metaphysis in two of these. In three cases it was in the upper epiphysis of a tibia, involving the adjacent metaphysis in two again. In one case it was in the lower epiphysis and adjacent metaphysis of a tibia. In still another case it involved the tuberosities, part of the capital epiphysis and the adjacent portion of the metaphysis of a humerus.

In connection with localization, it remains to be noted that in all of the nine cases reported by Codman [3] the lesion was in the upper end of a humerus. Indeed, such references to this lesion as exist in textbooks and articles sometimes include the statement that there is predi-

picture and, particularly, mention of the multinuclear giant cells, to which so much importance has been attached in the past. It is true that multinuclear giant cells are to be found, either singly or in small collections, here and there in the lesion, but most of them are seen around areas of hemorrhage, necrosis and reconstruction and we think they should hence not be regarded as part of the primary pattern of the lesion.

It was Ewing [2] who denoted as the "calcifying giant cell tumor" the lesion we are discussing here. He was certainly correct in pointing out that it was not an ordinary giant cell tumor, and that the lesion is a benign one though it has often been mistaken for a malignant one and specifically for an osteogenic sarcoma. However, it was Codman [3] who presented what seems to be the first detailed discussion of the lesion. His paper, entitled "Epiphyseal Chondromatous Giant Cell Tumors of the Upper End of the Humerus," was based on nine cases culled from the Registry of Bone Sarcoma. Codman, too, was led by clinical, roentgenographic and anatomic uniformities to regard the lesion as an entity, though like Ewing he held that this entity belonged within the category of giant cell tumor. Again like Ewing, Codman stressed the predilection of the lesion for the upper end of the humerus and specifically for the vicinity of its tuberosities. However, he did not believe that it was necessarily limited to this region, and, in a more recent reference to the lesion, Ewing [4] also expressed this opinion and seems to imply that it ought no longer to be considered as a form of giant cell tumor.

Otherwise the literature on the subject is very meager. One of the cases included in Codman's [3] report had previously been reported by Geschickter and Copeland [5] as a chondroblastic sarcoma.* Another had been reported by Phemister [6] as a chondrosarcoma. There can be no doubt that the case which King [7] reported as "an example of benign osteogenic sarcoma" likewise represents an instance of the lesion.

We ourselves have studied nine examples of the condition from the records of our laboratory, and these cases, with the pertinent clinical data, constitute the foundation of the present discussion. Like Codman,[3] we find that the condition is a very distinctive one, that the

* Since submitting the manuscript for publication, we have come across an instance of the lesion in the upper end of a tibia which Hammarström,[5a] too, reported as a chondroblastic sarcoma. This case was subsequently re-evaluated by Bergstrand [5b] (from whose laboratory it originally came). He felt that the original designation "chondroblastic sarcoma" was incorrect and thought that the tumor represented a vascular tissue growth but did not state whether or not he still regarded the lesion as malignant. On the other hand, Copeland [5c] continues to refer to the lesion as a chondroblastic sarcoma, and specifically as "a rare osteolytic variant of osteogenic sarcoma, sometimes erroneously called cartilaginous giant cell tumor."

into the joint, through the region of the intercondyloid fossa. In the case described by King,[7] an illustration showed the tumor presenting between the condyles of the femur, the tumor having its origin in the outer condyle. In connection with the question of extension of the tumor to the articular surface of the affected epiphysis, it may be well to call attention to Codman's [3] statement that the tumor does not at any point destroy the bone to the very cartilage. His statement is based upon findings (mainly roentgenographic) in respect to lesions in the upper end of the humerus and particularly those developing in the vicinity of the tubercles. Nevertheless, in the resected specimen from a lesion in this region we found that the tumor had invaded the capital epiphysis near the tubercle and actually extended to the deep surface of the articular cartilage, causing the latter to bulge over a small area and even destroying it in part.

Clinical Complaints

In our cases, the complaints had been present for 3 months to 1 year before medical aid was sought. Some of the patients gave a history of an injury—usually a fall or twist—which they connected with the condition in some way, while others gave no history of any possibly relevant injury. All related their complaints to the affected joint region, which was generally reported to be painful, more or less swollen and limited in motion. Examination usually revealed also some increase of local heat, a point of tenderness, limping when a lower extremity was concerned and even some flexion deformity, in association with local muscular atrophy. Slight hydroarthrosis, too, was discovered in some cases.

In relation to the upper end of the humerus, the lesion has become at least somewhat familiar clinically, and is likely to be correctly diagnosed even without tissue examination, by those who know it. When it is not, it is most likely to be clinically considered a chondrosarcoma or an osteogenic sarcoma. A lesion in the knee region may be misdiagnosed clinically as tuberculosis. On the other hand, if a neoplasm is considered, it is most often thought to be a giant cell tumor. Actually, the relative smallness of the lesion and its mottled, spotty appearance in the roentgenogram (aside from the youthfulness of the subjects), should promptly rule out giant cell tumor.[1]

Treatment

As noted, except in relation to the shoulder region, cases of benign chondroblastoma of bone are not yet usually diagnosed clinically. Thorough curettement of the lesion appears to be the method of choice,

lection for this region and, perhaps, even that it is found in it alone. Codman did not think it necessarily had such a restricted localization, but he did think that that localization was characteristic for it. As already indicated, our experience does not confirm this idea. There can be no doubt that when Codman was culling his cases from the Registry of Bone Sarcoma he was concentrating his attention particularly upon the shoulder region, in accordance with his special interest. However, he brings out a useful point in regard to localization in the upper end of the humerus, namely, the prominence of involvement of the tubercles * and the sparing or limited involvement of the capital epiphysis.

Roentgenographic Appearance

In roentgenograms (Figs. 1 to 6), the tumor is oval or round, and, as noted, may be restricted to part of an epiphysis or involve also part of the adjacent metaphysis. Here the lesion may be so eccentrically located as to bulge out the cortex. Those lesions which are restricted to the epiphysis are smaller, as a rule, than those involving the adjacent metaphysis as well. In neither instance does the lesion attain a very large size. In our cases, the maximum diameter usually ranged between 3 and 6 cm., though we had two cases in which it was only about 1 cm.

The lesion appears as a fuzzily rarefied and even mottled focus which tends to be delimited by a rather well defined, narrow, encircling line from the neighboring bone. It may appear somewhat trabeculated in addition, especially when viewed from certain angles. The fuzzy, mottled appearance of the tumor reflects the calcification that is going on unevenly within it. Beyond the limits of the tumor, but not necessarily evenly around the lesion, a smaller or larger area of the bone may appear densified.

Toward the joint surface, the lesion may, and frequently does, at some point, extend to the articular cartilage, and may even bulge into the joint. Thus in one of our cases in which the tumor was in the upper epiphysis of a tibia, it had expanded the intercondyloid eminence and led to deformity and protrusion of the tibial spine. In two of our cases, a tumor in a lower femoral condyle also protruded forward

* The greater tubercle of the humerus nearly always develops from an ossification center separate from that of the head proper. This separate center may appear between the 8th and 12th months and has almost always appeared by the 21st month if it is destined to develop at all. The commonest time of union of this epiphysis with that of the head is in the 7th year, but it may take place at any other time between the 4th and 12th years. Whether a center of ossification separate from that of the head develops for the lesser tubercle has not yet become clearly established, and even those who maintain that it does are not consistent as to the chronology of its appearance and of its fusion with the head.

all clinical complaints, and a 3-year follow-up found the patient entirely well and free from evidences of recurrence. In the other case (that represented by the specimen found in the Erdheim collection) no follow-up data are available. The follow-up histories in Codman's [3] nine cases showed all the patients well 3 to 10 years after coming under treatment.

Pathology

Gross Pathology

While we were able to examine pathologic material from all our cases, only in two did we have the opportunity of seeing the lesion intact in its setting. In one instance, the tumor was a rather small one in a femoral condyle and appeared as a small, friable mass of grayish tissue. In the other case,* the tumor was in the upper end of a humerus and involved the tubercles, metaphysis and an adjacent bit of the capital epiphysis. In this specimen, the articular cartilage of the humeral head was found preserved almost throughout. Along its posterior border it showed a chain of small marginal exostoses. In addition, on the dome of the head, there was a bumpy protrusion of the cartilage, caused (as was evident from subsequent sectioning) by a subchondral hemorrhagic cystic cavity in the tumor. Most of the articular cartilage over this bump was still intact, but posteriorly the cartilage was defective over a small area and a thin layer of connective tissue filled the base of the defect.

A coronal section through the specimen (Fig. 7) showed that while laterally the tumor affected the region of the tuberosities and extended into the capital epiphysis (to the articular cartilage) and into the metaphysis, medially parts of both the capital epiphysis and the metaphysis were uninvolved. In the cut section, the tumor measured 6 cm. in its long axis and 4 cm. across. The tumor tissue was sharply delimited from the neighboring uninvolved bone and the delimiting margin was convexly lobulated. Such tumor tissue as was not cystic or hemorrhagic (as it was in fully two-thirds of the lesion) was soft but rather firm and in some places even gritty and of a gray-yellow or gray-brown color. Nowhere in the tumor could one note grossly any tissue which could indubitably be regarded as bone. However, within the tumor and quite enclosed by tumor tissue but displaced from their normal position, one could note residual islands of epiphysial cartilage plate. The cystic and hemorrhagic areas were disposed mainly about the periphery. The cysts were 1 cm. or less in diameter and were outlined by a thin

* We found the specimen (catalogued as a sarcoma) in the collection of pathologic material preserved by the late Viennese pathologist, Jacob Erdheim, now housed in the laboratory of our hospital.

and is indeed likely to have been done before the diagnosis has been established on a histologic basis. In those of our cases in which this treatment was carried out, the lesion healed without recurrence, and function was restored. The end results in these cases were the same, whether or not the thorough curettement had been followed by moderate radiation therapy. On the other hand, in a case in which the lesion was in a femur and in which merely a punch biopsy was taken and the lesion subsequently treated with heavy irradiation, a contraction of the knee resulted, requiring arthrodesis for correction. Furthermore, in this case, islands of viable tumor tissue were still recognizable in the articular shavings removed in the course of the operative correction. It is true that Codman [3] cited two cases in which healing followed treatment by irradiation alone, but the significance of these cases is not clear, since the diagnosis was based solely upon the clinical findings and not even a punch biopsy specimen was taken. Altogether, we know that *thorough* curettage alone is sufficient to cure these lesions, and we see no necessity for using irradiation, either alone or as an adjuvant to curettage. On the whole, the weight of evidence seems to be against the necessity of irradiation and is certainly against very vigorous irradiation, either with or without previous curettage.

Follow-Up

In one case still under observation, only 9 months after an incomplete curettage of the lesion, the patient is entirely free of clinical complaints and the roentgenograms show substantial recalcification of the affected area. In another case, an incomplete removal was likewise followed by evidence of rapid calcification at the site of the lesion, and the clinical complaints disappeared within 5 months, but no further information on the subsequent history is available. In three cases the patients were discharged from the outpatient department as cured 1 year after a thorough curettage, since they no longer presented any complaints clinically and there was satisfactory ossification at the site of the lesion roentgenographically. None of these three patients has returned, and for two of them it is now 12 years since their treatment at the hospital was completed. Two cases in which, after a preliminary biopsy, the lesion was treated by heavy irradiation, have been followed for 5 years, and we know that in these there has been no recurrence of the lesion, though the neighboring joint (a knee in both instances) became stiff, requiring, as noted above, an arthrodesis in one case, because of contracture. In two instances the lesion was completely resected. In one of these, in which the lesion was a small one in the condyle of a femur, resection was followed by prompt disappearance of

tion detectable at the synapse, but not completely as to the characteristics of the degenerative change. Phalen and Davenport [29] inclined to the belief that the variability in appearance at the synapse precludes the use of morphology as a criterion of degeneration. Barnard [9] failed to find progressive degenerative changes after appropriate experimental manipulation. He found, however, that boutons diminish in number following section of the axons of the cells about which they terminate (retrograde effect). This "axon reaction" is denied in the experimental work of Lawrentjew,[3] Barr [30] and Schadewald.[31]

The change in the terminals around cells of the nucleus dorsalis in the presence of an irritative lesion at the dorsal roots (Table XV, acute meningitis), and the diminution in number of terminals about cells of the dentate nucleus with loss of Purkinje cells (Table XVI) support the conclusion that alteration in boutons at the synapse is related to damage of the parent cell body or axon (progressive). By no means, however, is it implied that this alteration must, in every case, be considered degenerative. On the contrary, the alteration in boutons seems to be related frequently to increased synaptic activity, *e.g.*, increased number of terminals (beading effect) in early poliomyelitis (Table XV).

Data from case 53 in the previous report [11] indicated that axon reaction was absent around cells of the posterolateral group in the lumbar enlargement 5 months after amputation of the leg of the same side. Case H16, amputation of left leg 26 years previously, likewise showed no differences in counts of terminals around cells of appropriate columns of both sides in the lumbar enlargement. Further evidence that axon reaction appears to be absent in the human central nervous system is presented in Figures 4 and 5. The photomicrograph in Figure 4 shows persistent basket terminals at the sites of degenerated Purkinje cells in case H115 whose symptoms of Purkinje cell loss existed for 18 years (Table I). Figure 5 shows boutons adjacent to an anterior horn cell undergoing neuronophagia in a case of poliomyelitis (H106). Certain of these terminals are altered morphologically but it would seem this must be related to inflammatory effect on the cells or fibers supplying the afferent components of the synapse. To check this further, cells have been counted according to their appearance (uninvolved, damaged, or degenerated) at the time of examination in seven cases of poliomyelitis, and the nature of the terminals ending in relation to the cells noted (Table XVII). It is evident from the data that the alterations in the terminals are unrelated to the condition of the receptive component about which they end. Some terminals appeared to be engulfed by the neuronophagic cells, which may account

lary and confined to the entering fibers (acute cases) the terminals adjacent to the cells of the nucleus dorsalis were altered morphologically but did not seem to be diminished in number (actually increased); on the other hand, in the subacute cases (with intramedullary pathology) there appeared to be a diminution in number of terminals with approximately the same proportion of remaining terminals altered morphologically.

An opportunity to observe terminal effects from loss of cells with relatively short axons was afforded by the three cases of parenchymatous cortical cerebellar atrophy (H113, H114, H115). The pathologic manifestations in this disease include the disappearance of Purkinje cells from the cerebellar cortex with persistence of the basket terminals identifying the positions of degenerated cells (Parker and Kernohan[26]). In Table XVI data from these cases are compared with data from

TABLE XVI

Purkinje Cell Counts in the Cerebellar Cortex Correlated with Terminal Counts in the Dentate Nucleus of the Same Side

	Purkinje layer			Dentate nucleus			
	Normal cell sections	Degenerated cell sections	Per cent cells lost	Cell sections	Boutons	Boutons per cell section	Per cent boutons lost
Control (3 uninvolved)	150	0	0%	100	130	1.30	0%
Parenchymatous cortical cerebellar atrophy (3 cases)	41	261	86%	300	142	0.47	62%

uninvolved cases. It appears, assuming that all terminals ending around cells of the dentate nucleus are from Purkinje cells, that loss of Purkinje cells is reflected in the diminished number of boutons ending in relation to cells of the dentate nucleus. It is possible that all of the terminals of these cells do not disappear with degeneration of the cells (see Cajal,[2] for persistence of axon parts after section). Purkinje cell counts from flocculus and inferior vermis were excluded since the terminals of these cells probably end in relation to cells of the cerebellar roof nuclei.

8. PROGRESSIVE AND RETROGRADE EFFECTS AT THE SYNAPSE

That changes in boutons occur when their associated axons have been damaged experimentally is concluded from the work of Lawrentjew,[3] Gibson,[4] Hoff,[7,8] Foerster, Gagel and Sheehan,[21] Schimert,[22] Snider,[23] Hoff and Hoff[27] and Fulton, Hoff and Kennard.[28] These authors have agreed on the general principle of progressive degenera-

terminals do not appear to be related to the condition of the cells about which they end. With the supposition, then, that all bouton changes are related to influence on the parent cell or axon it is interesting to note the condition of the cells in the various columns and translate this to synaptic effect. Of 449 anterior horn cells counted in 7 cases of poliomyelitis, 310 (69 per cent) were damaged or degenerated. On the basis of the above premise the bouton effect from this injury should be evident at the peripheral end-plates (Carey [5]). Of 367 sensory and internuncial cells counted in the same series, 122 (33 per cent) appeared damaged or degenerated. In this series an average of 44 per cent of the terminals adjacent to anterior horn cells appeared granular (Table XVIII). This represents an increase of approximately 36 per cent in granular forms in this location over the control averages (Text-Figs. 1 and 3). The similarity between the proportion of damaged sensory and internuncial cells (33 per cent) and the proportion of altered terminals adjacent to cells of the anterior horn (36 per cent) suggests that most of the boutons in relation to efferent cells have their source in internuncial columns. An inflammatory process of the nature of poliomyelitis might affect axis cylinders and their ramifications without observable change in the distant parent cell body. Hurst [24] has demonstrated granulation of neurofibrillae of unknown sources in experimental poliomyelitis. In the present material, granularity of neurofibrillae, appearing as a beading effect, was common in the neuropil in inflammatory cases.

The 3 cases (H70, H99, H111) of old poliomyelitis are of interest chiefly in demonstrating the persistence of bouton formations adjacent to glial scars identifying the position of degenerated cells. In cases H70 and H111 in which the condition had existed for 73 and 50 years, respectively, the involved side stained lightly and the cell scars were difficult to identify with certainty. In case H99, however, the scars were prominent (presumably glial replacement) and around 32 of these scars, 58 boutons have been identified.

(*b*) *Inflammatory Myelitides Other Than Poliomyelitis*

This group includes cases which are fundamentally similar to the above although they vary in etiology and in certain histologic details. Included are Landry's paralysis (H5), hemorrhagic encephalomyelitis (after Baker [32]) (H107), postinfectious encephalomyelitis (H43), postmeasles encephalitis (H109), actinomycotic myelitis (H89) and abscess (H72). Histologically, these cases were all inflammatory and many showed the neurofibrillar beading effect described for poliomyelitis. The bouton distributions for these cases are listed in Table XIX.

for the slight diminution in the number of boutons per cell section around degenerated cells. It is concluded that retrograde transsynaptic effect is not demonstrable in human material with the technic used in the present study.

9. INDIVIDUAL CASE DATA

The data in Table XIV indicate that quantitative differences in both the distribution of bouton configurations and the number of terminals per cell section appear when cases are grouped according to common pathogenesis. These data represent the total average of the synaptic effects across an entire section and, in so doing, fall short of an accurate description of the pattern of synaptic change which, in many instances, appears to be peculiar to certain diseases. In the descriptions to follow the diseases will be grouped, in so far as possible, according to common alterations at the synapse. Text-Figures 1 and 2, representing the regional distribution (percentage of granular forms) in nonneuropathologic cases under and over 30 years of age, are control graphs for the comparisons to follow.

TABLE XVII

Comparisons Illustrating Absence of Retrograde Transsynaptic Effect at the Human Synapse

Appearance of cell section	Number of cell sections	Number of boutons	Per cent granular boutons	Boutons per cell section
Uninvolved	384	1466	35.61	3.82
Damaged	216	817	39.41	3.78
Degenerated	216	762	39.37	3.53

(*a*) *Poliomyelitis*

The counts in eight cases of poliomyelitis are listed by region and nuclear column in Table XVIII. The essential data for each case may be obtained from Table I. It will be noted that there is considerable variation from case to case. Certain circumstances suggest that these differences may be related to position of the lesions. For example, there was a relatively large number (49.70 per cent) of altered terminals on the cells of the sensory and internuncial groups in case H106 which was peculiar in having an involvement of the dorsal root ganglia (Table I). The general pattern of morphologic change at the synapse in poliomyelitis is represented by the graph (Text-Fig. 3) of case H108 from Table XVIII. Since the cases in this series are under 30 years of age they may be compared with Text-Figure 1.

It has been mentioned (Table XVII) that the alterations in the

The variations in the group are extreme, and it will be noted by comparing with Table XVIII that only cases H5 (Landry's paralysis), H43 (postinfectious encephalomyelitis) and H109 (postmeasles encephalitis) exhibited a synapse pattern similar to poliomyelitis. The synapses of case H107 (hemorrhagic encephalomyelitis) appeared to be affected primarily in the dorsal horn. Cases H89 and H72 (bacterial myelitides) showed extreme necrosis in cord sections. It is interesting to note the apparent increase in number of terminals ending in relation to the somatic efferent cells in cases H5, H43 and H109. Of 147 internuncial cells counted in these cases, only 4 were completely degenerated at the time of observation while 81 were demonstrably altered (fragmented neurofibrillae, eccentric nuclei, swelling). In these cases the beading effect in neurofibrillae of the neuropil was prominent, suggesting that the apparent increase in contact points at the ventral horn synapses may be a peripheral manifestation of beading along the parent stem. Case H109 had a concomitant meningeal exudate which may account for the relatively large proportion of granular boutons on the cells of the dorsal horn (see Meningitis). The proportionate distribution of terminal forms appearing in H5 (Landry's paralysis) is illustrated graphically in Text-Figure 4. The total averages for the counts in the cases of inflammatory myelitis are included in Table XIV.

(*c*) *Meningitis*

Certain differences in the synapses within this group have been pointed out (Table XV, second part). Counts of the individual cases are listed in Table XX. The averages for the entire group indicate that the predominant synaptic effect was on the terminals of the dorsal horn (Fig. 3, Plate 165, taken from case H7). The central reactions were obviously less marked in the group with no purulent exudates (H20, H27, H71), and the effect in the dorsal horn appeared to be diminished somewhat in case H84 in which continuous drainage had been instituted. In keeping with the previous statement that terminal change is related to influence on the parent stem, the assumption is that the meningeal exudate is responsible for much of the intramedullary change in the cases of this group. Such influence should be manifest only on the terminals of the dorsal root fibers intramedullarly, since irritation of the ventral roots would not effect an alteration transsynaptically. It has been mentioned that many of the cases of purulent meningitis, particularly those persisting for several weeks, show intramedullary lesions of an inflammatory nature. Excluding cases H7 and H53 and the chronic meningitides, 467 cells have been counted, 231 or 49 per cent of which are pathologically altered. Obviously, the synaptic pattern in these

TABLE XVIII

Bouton Distribution in Poliomyelitis

Case	Region*	Sensory and internuncial				Nucleus dorsalis				Intermedio-lateral				Somatic efferent			
		Cell sections	Boutons	Per cent granular	Number per cell section	Cell sections	Boutons	Per cent granular	Number per cell section	Cell sections	Boutons	Per cent granular	Number per cell section	Cell sections	Boutons	Per cent granular	Number per cell section
H94	L	47	125	30.40	2.66									55	341	36.07	6.20
H108	T	36	13	30.74	0.36	22	23	39.12	1.05	28	22	31.82	0.78	59	80	68.75	1.36
H41	T	20	55	36.36	2.75	8	20	60.00	2.50	6	25	40.00	4.17	25	146	58.90	5.84
H110	L	60	60	21.66	1.00									70	584	42.46	8.34
H106	S	47	167	49.70	3.55									64	316	37.34	4.94
H90	C	58	70	44.29	1.21									39	252	52.38	6.46
H101	L	58	187	25.67	3.22									88	338	34.02	3.84
H95	L	39	118	22.03	3.03									46	349	26.93	7.59

* L = lumbar; T = thoracic; S = sacral; C = upper cervical.

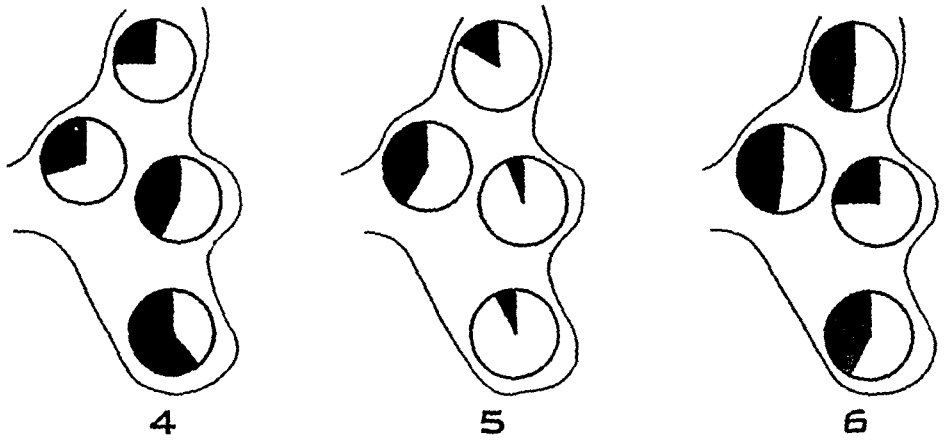
TABLE XIX

Bouton Distribution in Inflammatory Myelitis Other Than Poliomyelitis

Case	Region*	Sensory and internuncial				Nucleus dorsalis				Intermedio-lateral				Somatic efferent			
		Cell sections	Boutons	Per cent granular	Number per cell section	Cell sections	Boutons	Per cent granular	Number per cell section	Cell sections	Boutons	Per cent granular	Number per cell section	Cell sections	Boutons	Per cent granular	Number per cell section
H5	T	44	126	25.39	2.86	18	67	28.35	3.72	20	103	43.68	5.15	55	560	62.85	10.18
H43	T	27	66	25.75	2.44	12	52	25.00	4.33	12	29	17.24	2.42	35	526	41.44	15.02
H43	L	17	59	25.42	3.47	8	54	12.96	6.75					22	350	43.14	15.90
H109	L	59	205	55.12	3.47									55	564	37.76	10.25
H89	T	13	17	52.94	1.31	17	30	23.33	1.76	25	18	50.00	0.72	22	120	60.83	5.45
H72	C	23	5	60.00	0.22									33	18	72.22	0.55
H107	L	34	137	48.17	4.02									43	212	18.38	4.93

* T = thoracic; L = lumbar; C = upper cervical.

cases must be considered that of a combined meningitis and myelitis (compare with case H109, Table XIX). An apparent increase in the number of terminals (beading effect) was evident in certain of these purulent lesions (H84, H85) which had not persisted over 3 weeks (Table XV). Cases H7 and H53 showed little intramedullary alteration. The distribution of granular forms of terminals in case H7 is shown in Text-Figure 5. This was a case of purulent meningitis with a minimum of intramedullary pathology. The pattern in case H102, presenting combined meningitis and myelitis effects, is shown in Text-Figure 6 (compare Text-Fig. 5 with Text-Fig. 1, and Text-Fig. 6 with Text-Fig. 2).



TEXT-FIGURE 4. Granular bouton distribution in a thoracic field of case H5, Landry's paralysis. (See Text-Fig. 1 for further explanation.)

TEXT-FIGURE 5. Synaptic pattern of a thoracic field in case H7, purulent meningitis.

TEXT-FIGURE 6. Synaptic pattern of a thoracic field in case H102, meningeomyelitis.

Case H84 is of particular interest in that the meningeal exudate had been drained from the lumbar region. In the thoracic section counted (Table XX), very little exudate was in evidence and the greater synaptic effect appeared on cells of the somatic efferent group. At this level 95 cells were counted and only 1 was demonstrably pathologic. In the cervical section of the same cord, 81 cells were counted and 58 of these were undergoing pathologic change. This suggests a regional relationship between exudate and underlying intramedullary pathology. Further, the diminished change in the terminals around cells of the nucleus dorsalis relative to alteration around cells of the sensory and internuncial groups (compare with others in the same series) suggests a separate source for the boutons of these two columns. The coincidence of absence of exudate at the lumbar enlargement appears to support the contention that the nucleus dorsalis is, at least in part, a receptive group for fibers entering in that lower region. Alterations at the thoracic anterior horn synapses may be related to the intramedul-

TABLE XX

Bouton Distribution in Meningitis

Case	Region*	Sensory and internuncial				Nucleus dorsalis				Intermedio-lateral				Somatic efferent			
		Cell sections	Boutons	Per cent granular	Number per cell section	Cell sections	Boutons	Per cent granular	Number per cell section	Cell sections	Boutons	Per cent granular	Number per cell section	Cell sections	Boutons	Per cent granular	Number per cell section
H84	T	21	94	34.04	4.47	27	90	32.22	3.33	22	24	16.67	1.09	25	258	44.96	10.32
H84	C	46	279	56.63	6.06									35	491	48.88	14.02
H85	L	19	145	56.55	7.63	6	188	60.63	31.33					23	535	35.51	23.26
H93	T	14	53	56.61	3.78	23	40	60.00	1.74	18	32	28.12	1.77	26	124	30.64	4.77
H7	T	116	354	16.67	3.05	74	678	47.93	9.16	20	56	5.35	2.80	92	716	6.84	7.78
H53	T	38	64	32.81	1.68	28	61	45.90	2.18	8	14	21.43	1.75	52	223	13.90	4.29
H102	T	44	113	48.67	2.56	26	51	49.02	1.96	24	24	25.00	1.00	44	182	42.30	4.14
H87	T	20	25	48.00	1.27	17	67	44.78	3.95	35	41	26.83	1.15	35	138	44.93	3.94
H98	T	25	29	37.92	1.16	22	49	48.97	2.22	18	17	23.52	0.94	28	63	52.38	2.25
H20	T	23	53	24.53	2.30	19	114	20.56	6.00	6	14	7.14	2.33	24	137	13.87	5.70
H27	T	18	50	8.00	2.78	9	72	25.00	8.00	7	23	39.13	3.29	18	138	22.46	7.66
H71	T	34	91	40.66	2.68	17	155	52.91	9.12	12	17	23.53	1.42	28	219	36.07	7.82

* T = thoracic; C = upper cervical; L = lumbar.

TABLE XXI

Bouton Distribution in Cords Which Have Been Directly Traumatized or Invaded by Tumor

Case	Position of lesion*	Region examined*	Sensory and internuncial				Nucleus dorsalis				Intermedio-lateral				Somatic efferent			
			Cell sections	Boutons	Per cent granular	Number per cell section	Cell sections	Boutons	Per cent granular	Number per cell section	Cell sections	Boutons	Per cent granular	Number per cell section	Cell sections	Boutons	Per cent granular	Number per cell section
H49	C vert.	Ce	24	109	41.28	4.54									32	126	46.82	3.94
H64	cauda equina	L	33	10	40.00	0.30									50	34	70.58	0.68
H64	cauda equina	T	27	52	67.31	1.93	20	72	65.28	3.60	17	32	18.75	1.88	28	87	63.22	3.11
H64	cauda equina	Ce	34	138	60.87	4.06									67	463	53.77	6.91
H64	cauda equina	C	35	106	41.48	3.03									50	282	50.34	5.64
H50	T7-Co 1	T12	31	31	74.19	1.00	22	21	80.95	0.95	2	11	72.72	0.92	31	37	78.37	1.19
H50	T7-Co 1	T7									5	5	60.00	1.00	21	167	43.11	7.95
H50	T7-Co 1	Ce	33	70	34.28	2.12									39	386	39.63	9.89
H50	T7-Co 1	C	50	66	45.45	1.32									54	246	50.40	4.55
H1	C6	T4	49	190	43.16	3.88	24	140	42.23	5.83	30	72	58.33	2.40	38	591	63.46	15.5
H176	T9-10	Ce	26	70	55.71	2.69									56	334	36.53	5.96
H175	C	Ce	25	32	56.25	1.28									36	152	50.64	4.22
H183	C5	Ce	16	31	54.84	1.94									26	126	53.17	4.84
H91	C	T	24	104	57.69	4.33	12	79	41.77	6.58	17	49	26.53	2.88	25	281	50.17	11.24

* C = upper cervical; T = thoracic; Co = coccygeal; Ce = cervical enlargement; L = lumbar.

lary damage at higher levels. The absence of marked synaptic change at the somatic efferent cells in case H7 (Text-Fig. 5) is considered to suggest that few, if any, dorsal root fibers pass directly to the ventral horn cells. This supports the contention mentioned above that internuncial cells seem to be the main source of the distributing components to the final-common-path cells. The evidence for this from group data in cases of meningitis is not striking due to the common presence of intramedullary pathology.[8, 9, 27, 28]

(*d*). *Direct Cord Injury* (*Trauma and Tumor*)

This group of cases includes those in which the spinal cord had been traumatized or invaded in such a manner as to establish a definite lesion. The data from the cases are listed in Table XXI together with the position of the lesions and the regions counted. Case H49 was that of a carcinoma of the lung with metastases to the upper thoracic and cervical vertebrae. There appeared to be no encroachment on the cord itself, the damage probably resulting from involvement of radicular and epidural vasculature. There was uniform, diffuse demyelination throughout the cervical cord. The significant lesion in case H64 was tumor erosion of the lower four lumbar vertebrae with involvement of the cauda and conus but avoiding the spinal cord proper. Weigert preparations showed total demyelination of the dorsal columns at the level of the lumbar enlargement. Case H50 was one of multiple myeloma with destruction of vertebrae T7 and T8 and softening of the cord below this level and partial destruction above up to cord level T7.

It would seem from the data that any conception of a definitely localized traumatic or tumor lesion in the human cord is erroneous. In addition to the generalized synaptic effect in the three tumor cases (H49, H64, H50) it is interesting to note the cellular condition in these cases. Thirty-nine of 56 cells counted in case H49 were damaged. Of 101 cells counted in a section through the cervical enlargement of case H64, 54 were demonstrably altered pathologically. The predominant manifestation at this distant focus was marked vacuolization of the nerve cells. In an upper cervical field from case H50, 35 of 104 cells appeared damaged. In addition to widespread cellular response to these focal lesions, both the neuropil of the gray matter and the neuraxes of the white matter showed marked alteration. Prominent among the changes in these locations were swelling of the neuraxes with some fragmentation and clubbing of the fragmented ends. The beading effect, described for neurofibrillae in inflammatory involvement, was absent in these cases. Lumbar and sacral sections from case H50 were completely necrotic, only 38 degenerated cells with 43 boutons being

way, the sources of which were obscure. It is interesting to note that terminals, obviously of afferent or internuncial sources, were demonstrable despite the absence of a cerebral cortex, which undoubtedly has much to do with the development of interneuronal connections.

H42 was a postoperative case of right cerebellar tumor (ependymoma). The count from the right field of a thoracic section is given in Table XXII. A purulent meningitis was evident, clouding whatever retrograde effect might be present in the cord sections. The synaptic picture was that of a meningomyelitis (Table XX, and Text-Fig. 6).

The tumor (astrocytoma) in case H37 filled the third ventricle and extended laterally to obliterate the bodies and anterior horns of both lateral ventricles. It was attached superiorly to the corpus callosum and did not appear to involve other brain tissue. The bouton distribution at the thoracic level (Table XXII) compares favorably with the average of uninvolved cases of the same age group (Text-Fig. 1).

In case H29 an astrocytoma involved the left temporoparietal region at the posterior end of the lateral cerebral fissure. It extended to, but did not include, the basal ganglia. Comparison of the data from the left field of a thoracic cord section with the diagram of uninvolved controls in the same age group (Text-Fig. 2) indicates that no synaptic effect is evident at the level counted.

The tumor (glioblastoma multiforme) in case H45 was described as extending from the central sulcus to within 4 cm. of the right frontal pole and as involving only the anterior limb of the internal capsule. It would appear from the data in Table XXII that the efferent fibers from this area which gained the upper cervical cord (opposite side was counted) ended primarily in relation to cells of the anterior horn at this level. From the description of the tumor site it would seem to involve some of the motor cortex (but not the components which should be delivered to the upper cervical region) as well as most of the premotor cortex and additional frontal lobe. If this be true, it would seem that the effect demonstrated is related to the premotor involvement.[28] The cervical synapses are diagramed in Text-Figure 9.

In case H8 the tumor (glioblastoma multiforme) was described as involving the left superior temporal gyrus and the parietal operculum. From the site of the tumor it would appear to have involved the efferents from the lower part of the motor cortex. A count of the terminals on the cells of the opposite side in the cervical enlargement is given in Table XXII. The data indicate that terminals from this source ended primarily on cells of the sensory and internuncial columns. A sector diagram of the bouton distribution in the opposite field of a section through the cervical enlargement is shown in Text-Figure 10.

identifiable at these levels. Sections through the lower thoracic regions were necrotic with neurofibrillar granulation so pronounced as to cloud the certain identity of boutons. As compared with data in Table IV a diminished number of boutons appeared only at levels close to the lesion. This lends support to the conclusion previously stated that nerve terminals of long fibers may persist following degeneration of the parent cells.

Of the fracture cases, all but H76 presented similar pictures which are represented by the pattern in a thoracic section from case H1 in Text-Figure 7. H76 differed in that the lesion was below the region counted. The relatively greater proportion of granular forms of terminals around the dorsal horn cells in this case suggests that ascending fibers had a greater tendency to end in this region than descending fibers. A diagram of the pattern of synapses at the level counted in case H76 is shown in Text-Figure 8. The sections from the fracture cases showed swelling and clubbing of neuraxes similar to that described for the tumor cases, but in general, to a lesser extent.

(*e*) *The Cord Synapses with Cerebral Lesions*

The following cases demonstrate synaptic effects in the spinal cord as related to definitive lesions involving the intracranial structures. They differ from the previous group primarily in the distance of the lesion from the region observed. The counts from this group are presented in Table XXII. Essential data from the case histories may be found in Table I. On general inspection of the data in Table XXII it is evident that great variability existed in the group. Unlike the cases of cord tumor (Table XXI) the cerebral tumor cases did not seem to produce generalized alterations at the cord synapses. As a result it may be permissible to assume that these cases represent actual focal lesions, the effects of which at the cord synapses are due to direct involvement of the parent fibers or cells and not to general toxicity or vascular damage. The following discussions are based on this assumption.

Case H74 was one of hydranencephalus in which only 4 per cent (by weight) of forebrain tissue was present.[33] The boutons in this case (age 3 months) were demonstrated with pyridine silver. As a result their morphologic state cannot be compared with the remaining cases in the group. Certain aberrant characteristics were, however, apparent. The terminals were sparse in a dense pericellular net, and were uniformly small. No typical granular boutons were in evidence. Weigert and Marchi preparations were of little help in defining the tract effect in the cord at this age. Hematoxylin and eosin and silver preparations revealed an abundance of fibers in the region of the corticospinal path-

the spinal bouton distribution (cf. Text-Fig. 2). Although there was a definite cerebral lesion in this case, it might more properly be included in the group with general cerebral arteriosclerosis to be described below.

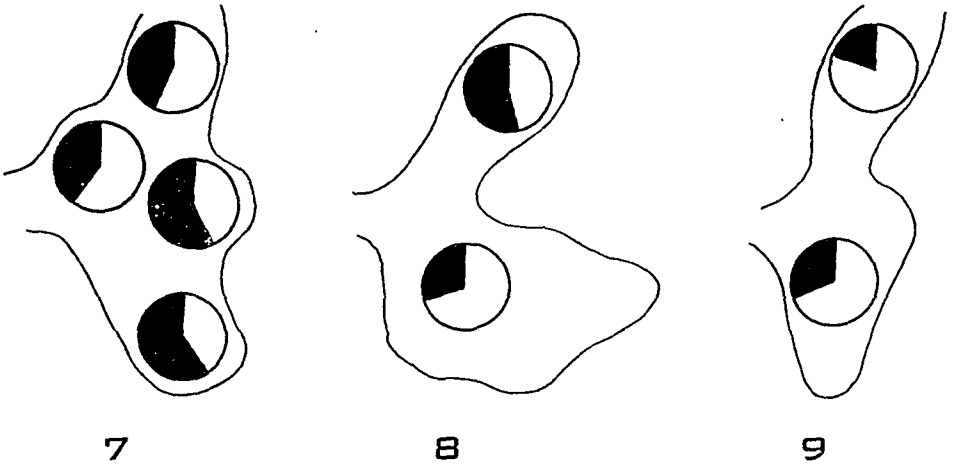
(*f*) *Toxic Myelitis*

This group includes cases in which involvement of the central nervous system is commonly considered to be due to toxic effects (Table XXIII). Case H82 was diagnosed clinically as encephalitis, but at autopsy as subacute bacterial endocarditis. The effect in case H58 (cystic hypothalamus) was undoubtedly thermal since the temperature rose to 110° F. following appendectomy. In view of the age of the latter patient (18 years), it seems evident that there was a slight bouton effect in hyperpyrexia. The same may be said of case H47 (uremia) in which there was generalized sepsis. The other case (H51), dying in uremia, from seminoma of the testicle with marked infiltration of both kidneys, compares favorably with the uninvolved control (Text-Fig. 1). It would seem, then, that the effect in case H47 was due more to sepsis than to uremia. Case H48 (age 47 years) showed a remarkably uninvolved synaptic picture for the age group (cf. Text-Fig. 2). It is interesting to compare the synaptic pattern of case H82 with those in Tables XVIII and XIX (encephalitis), especially since this case was diagnosed, clinically, as encephalitis. This suggests a relationship between the synaptic picture and the symptomatology. The intramedullary pathology in case H82 included satellitosis, some neuronophagia and slight beading of the neurofibrillae. Considering the age of the patient (20 years), this is a markedly altered picture of the synapses. Case H56 showed nothing unusual for the age group. The two cases of tetanus (H60, H112) were characterized by the reduction in the number of demonstrable boutons per cell section. Neither cord took the silver impregnation well, and the identity of terminals was obscured by the pronounced swelling of the nerve cells, and the granular appearance of the neuropil.

(*g*) *Inanition, Malnutrition, Metabolic Disturbances*

The data from these cases are listed in Table XXIV. With therapeutic measures available for administering food parenterally, it is difficult to obtain a case of frank inanition. Most representative of such a condition in the present series were cases H9 and H24, both with obstructive gastrointestinal lesions and both badly wasted at the time of death. Nothing unusual for the age group was in evidence at the cord synapses, however. Cases H11 and H44 were diabetic patients, the former dying from a cardiac lesion, the latter from pulmonary embolism

Case H17 had a hemorrhagic tumor (extragenital chorioepithelioma) involving the right temporal lobe with softening extending through the right basal ganglia and an additional tumor nodule in the left occipital pole. The right middle cerebral artery was thrombosed. A count of the boutons in a thoracic field of the left side is given in Table XXII. From the description of the lesion it is uncertain whether either limb of the internal capsule was involved. It is unlikely that striofugal fibers descended unbroken to so distant a point.



TEXT-FIGURE 7. Appearance of synapses in a thoracic field in case H1, transverse cervical traumatic myelitis. (See Text-Fig. 1 for further explanation.)

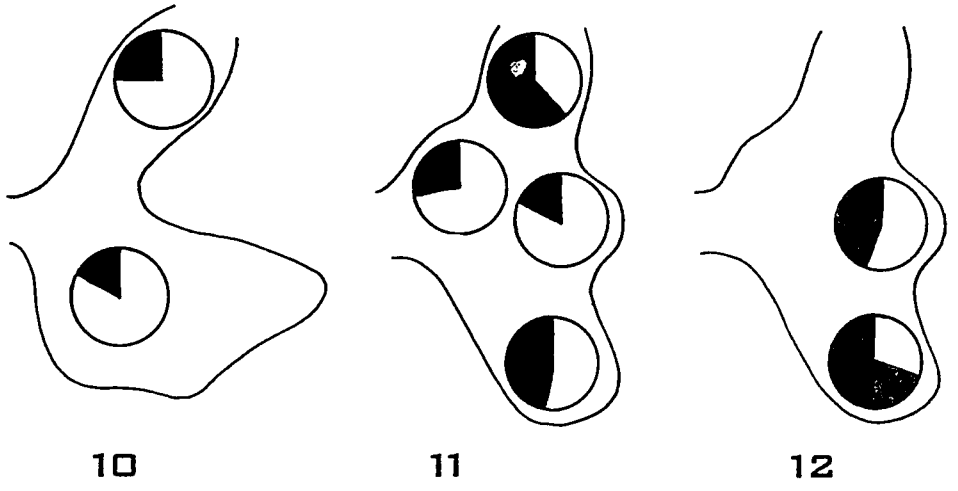
TEXT-FIGURE 8. Synaptic pattern in a field of the cervical enlargement in case H76, transverse thoracic traumatic myelitis. The posterior sector represents terminals on sensory and internuncial cells; the anterior sector, somatic efferent.

TEXT-FIGURE 9. Synaptic pattern in a left upper cervical field in case H45, right cerebral glioma involving premotor cortex and frontal lobe.

The tumor in H62 was an extensive right cerebral astrocytoma involving basal ganglia, both limbs of the internal capsule, and thalamus. The count from a left thoracic field is given in Table XXII and is represented diagrammatically in Text-Figure 11. Unlike cases H45 and H8 in which the lesions were apparently limited to one limb of the internal capsule, the effect on thoracic spinal boutons in this case appeared to be on cells of both the internuncial and somatic efferent groups. On the basis of the evidence from cases H45 and H8 it is possible that the alteration in the terminals around the internuncial groups in case H62 might be related to motor involvement and the changes in the boutons on the efferent cells related to a lesion of the premotor efferents.

Case H12 had a hemorrhage of the posterior right thalamus which extended into the right lateral ventricle. The brain vessels were sclerotic and there were evidences of previous hemorrhages. In view of the age of the patient (75 years) nothing may be concluded from

following laparotomy. Again, nothing unusual was in evidence at the spinal synapses. According to synaptic patterns, these last two might well be listed with the arteriosclerotic cases below and the first two with uninvolved cases in the older age group. Case H40 (insulin hypoglycemia) showed extensive alteration at the synapses. The lumbar section of this cord presented a picture similar to that described for hypoglycemia by Moersch and Kernohan.[34] Of 62 cells counted, 52 were pathologically altered and 8 completely degenerated. The general diffuse synaptic changes in the cases of malnutrition and deficiency suggest a general pathologic influence, similar in its synaptic manifestations to the myelitides (cf. Tables XVIII and XIX). This group in-



TEXT-FIGURE 10. Synaptic pattern in the right field of a section through the cervical enlargement in case H8, cerebral glioma involving left temporoparietal region. (See Text-Fig. 8 for further explanation.)

TEXT-FIGURE 11. Synaptic pattern in a left thoracic field in case H62, extensive right cerebral tumor. (See Text-Fig. 1 for further explanation.)

TEXT-FIGURE 12. Synaptic pattern in a thoracic field in case H103, necrosis of cord with obliteration of the dorsal horn at this level.

cludes cases H97, malnutrition; H96, vitamin D deficiency; H105, pellagra; H88, avitaminosis A; and H104, pigment degeneration. Of 360 cells counted in the group, 131 were altered pathologically and 5 were completely degenerated, showing a marked cellular, as well as synaptic, effect. Case H73 (questionably diagnosed as pernicious anemia, multiple sclerosis, or old toxic myelitis) showed almost completely degenerated gray matter with only a few shrunken nerve cells, about which were very few boutons. There was diffuse demyelination in the white matter, most prominent in the dorsal columns. The boutons were too few to warrant comment. Cases H100, H103 and H92 represented the disease described by Moersch and Kernohan [35] and Schunk and Kernohan [36] as acute or subacute progressive necrosis of the spinal cord, etiology unknown. In case H103 the dorsal horn was completely

TABLE XXII

Bouton Distribution in Spinal Cords With Associated Cerebral Lesions

Case	Region*	Sensory and internuncial				Nucleus dorsalis				Intermedio-lateral				Somatic efferent			
		Cell sections	Boutons	Per cent granular	Number per cell section	Cell sections	Boutons	Per cent granular	Number per cell section	Cell sections	Boutons	Per cent granular	Number per cell section	Cell sections	Boutons	Per cent granular	Number per cell section
H74	T	39	11		0.28	8	11		1.36	41	8		0.19	39	47		1.2
H42	T	37	48	14.58	1.3	27	54	29.63	2.00	9	8	25.00	0.89	27	181	21.00	6.7
H37	T	74	135	7.41	1.82	55	238	3.94	4.33	46	162	4.32	3.52	67	422	8.77	6.3
H29	T	67	267	29.96	3.98	41	232	22.22	5.71	19	102	15.69	5.36	60	330	26.67	5.5
H45	C	38	54	18.52	1.42									44	192	31.25	4.36
H18	Ce	63	169	24.26	2.68									60	425	17.18	7.08
H17	T	35	96	13.54	2.74	35	211	21.80	6.03	20	40	15.00	2.00	40	385	37.14	9.62
H62	T	20	111	61.26	5.55	19	60	28.33	3.15	11	23	17.39	2.09	15	165	47.87	11.00
H12	T	35	88	29.54	2.51	15	105	21.90	7.00	16	41	31.71	2.56	59	341	31.09	5.78
H3	T	35	99	18.18	2.83	40	240	22.08	6.00	25	65	23.08	2.60	62	463	23.33	7.46

* T = thoracic; C = upper cervical; Ce = cervical enlargement.

TABLE XXIII

Bouton Distribution at Cord Synapses Subject to Toxic Influence

Case	Region*	Sensory and internuncial				Nucleus dorsalis				Intermedio-lateral				Somatic efferent			
		Cell sections	Boutons	Per cent granular	Number per cell section	Cell sections	Boutons	Per cent granular	Number per cell section	Cell sections	Boutons	Per cent granular	Number per cell section	Cell sections	Boutons	Per cent granular	Number per cell section
H58	L	36	80	25.00	2.22	11	22	18.18	2.00	20	56	17.85	2.80	43	186	15.05	4.32
H48	T	39	79	8.87	2.02	41	106	13.21	2.58	22	35	11.43	1.59	31	131	20.33	4.22
H47	Ce	29	44	25.00	1.52	9	16	18.75	1.78					73	174	11.49	2.38
H51	S	75	101	7.92	1.35					8	23	8.69	2.87	59	281	5.33	4.76
H82	Ce	34	88	60.22	2.59									42	299	62.88	7.12
H56	T	30	124	29.83	4.13	22	117	29.91	5.32	12	44	20.45	3.67	48	200	20.50	4.17
H60	Ce	33	5	80.00	0.15									73	24	87.50	0.32
H112	L	44	6	100.00	0.13									66	17	88.23	0.25

* L = lumbar; T = thoracic; Ce = cervical enlargement; S = sacral.

TABLE XXV

Bouton Distribution in Arteriosclerosis

Case	Region*	Sensory and intermuncial				Nucleus dorsalis				Intermediolateral				Somatic efferent			
		Cell sections	Boutons	Per cent granular	Number per cell section	Cell sections	Boutons	Per cent granular	Number per cell section	Cell sections	Boutons	Per cent granular	Number per cell section	Cell sections	Boutons	Per cent granular	Number per cell section
H3	T	35	99	18.18	2.83	40	240	22.08	6.00	25	65	23.08	2.60	62	463	23.33	7.46
H6	L	75	238	13.87	3.17	30	288	12.72	7.60	14	29	6.89	2.07	62	574	15.67	9.26
H77	Ce	29	5	20.00	0.17									48	9	33.33	0.19
H81	L	21	116	28.96	5.52	16	74	17.57	4.63	13	35	20.00	2.69	14	311	36.01	22.21
H55	S	35	48	27.08	1.37	23	55	30.91	2.39	15	50	22.00	3.33	37	129	39.53	3.49
H23	T	25	85	14.12	2.43	25	157	16.56	6.28	15	47	14.89	3.13	36	197	18.78	5.47
H18	T	25	80	10.00	3.20									37	190	24.21	5.14
H31	T	35	112	25.89	3.20									50	385	16.36	7.70

*T = thoracic; L = lumbar; Ce = cervical enlargement; S = sacral.

necrotic at the level counted, presenting a condition similar to that in the thoracic section of case H50, Table XXI. These two sections were the only ones in the series which seemed to be specifically correlated with alteration of synapses in the intermediolateral column. H103 is represented diagrammatically in Text-Figure 12. H86 was a case of fracture followed by fat embolism. There was diffuse synaptic change.

(*h*) *Arteriosclerosis*

Clinical signs of involvement of the central nervous system were described in the histories of all cases in this group. None, however, showed any definite lesions at autopsy. The bouton counts for eight cases are listed in Table XXV. In general, the synaptic change was uniform and diffuse, although unexplained variations were evident among the boutons of the nuclear columns in some cases. Referring to Table XIV, it would seem these cases were not significantly different from the older age group of uninvolved cases.

SUMMARY

The relationships between distributing and receptive components at the central synapse may be altered demonstrably in the following ways: (1)

TABLE XXIV

Cord Bouton Distribution in Inanition, Malnutrition, Metabolic Disturbances, and Idiopathic Necrosis

Case	Region*	Sensory and internuncial				Nucleus dorsalis				Intermedio-lateral				Somatic efferent			
		Cell sections	Boutons	Per cent granular	Number per cell section	Cell sections	Boutons	Per cent granular	Number per cell section	Cell sections	Boutons	Per cent granular	Number per cell section	Cell sections	Boutons	Per cent granular	Number per cell section
H9	T	27	70	12.86	2.59	23	137	21.1	5.95	9	27	18.00	3.00	24	142	9.15	5.92
H24	T	41	99	20.20	2.41	9	50	26.00	5.55	24	53	30.19	2.21	23	161	17.39	7.00
H11	T	36	81	22.22	2.25	34	163	22.09	4.79	29	50	22.00	1.72	47	278	22.66	5.91
H44	T	29	63	23.81	2.17	40	206	23.70	5.15	27	56	21.43	2.07	39	177	23.17	4.54
H40	L	23	84	69.95	3.65									39	238	59.24	6.10
H97	T	18	18	16.66	1.00	10	41	36.57	4.10	19	15	20.00	0.79	36	216	35.64	6.00
H96	L	33	123	38.20	3.73									31	397	25.19	12.80
H105	L	31	50	24.00	1.61									51	200	25.00	3.92
H88	L	23	18	44.43	0.78									29	121	34.20	4.17
H104	T	24	23	21.73	0.96	16	27	25.92	1.68	15	20	25.00	1.34	24	202	34.16	8.41
H73	Ce	25	10	90.00	0.41									40	36	55.55	0.90
H100	T	31	32	37.50	1.03	13	26	50.00	2.00	9	10	30.00	1.11	28	79	53.16	2.82
H103	T									25	16	43.75	0.84	26	97	72.16	3.73
H92	T	14	8	37.50	0.57	12	9	22.22	0.75	6	9	44.45	1.50	28	55	40.00	1.96
H86	C	19	83	42.16	4.37									20	230	45.21	11.50

* T = thoracic; L = lumbar; Ce = cervical enlargement; C = upper cervical.

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damage to, or destruction of, the efferent component (cell body and its processes), which undoubtedly represents a total cancellation of synaptic function, in many cases probably reversible; (2) diminution in the number of terminals representing the distributing component (while this may affect synaptic transfer, there is no evidence that such a reduction operates to cancel it completely); (3) increase in the number of contact points at the synapse which is characteristic of inflammatory involvements and may reflect synaptic hyperfunction; (4) change in morphology of the afferent structures. The significance of this is obscure, but the following possibilities are suggested: (a) the altered boutons may represent a functional state; (b) they may represent degenerated terminals; (c) they may represent persistent terminals of degenerated axons.

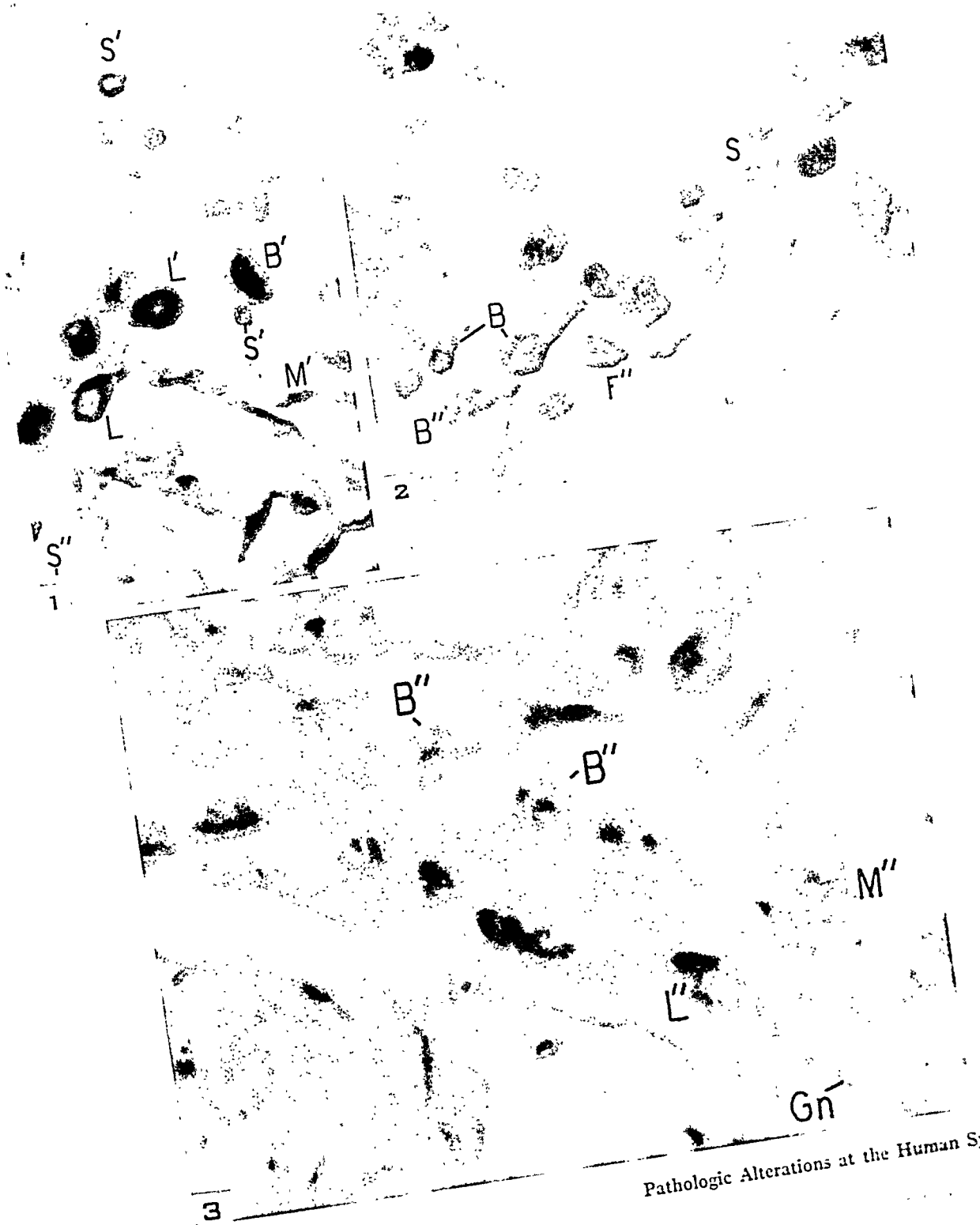
Of the factors evaluated for influence in bringing about these changes at the synapse, age and the presence of lesions are the most effective; technic and autolysis do not seem to be importantly related. The intensity of the alterations at the synapse depends upon the fundamental nature of the pathologic process, its duration and on the size of the cells (length of axons, particularly) involved. The manifestations of pathologic influence at the synapse vary with the nuclear group under observation, and certain alterative patterns appear to be peculiar to specific disease groups and to the location of definitive lesions. The observable bouton effects are related primarily to involvement of the parent structure. No retrograde, transsynaptic effect from damage to the efferent component is evident at the human central synapse.

NOTE: Acknowledgment is due Dr. J. W. Kernohan, Division of Neuropathology, Mayo Clinic, through whose courtesy thirty-three of the cases in the present study were made available. Dona Minckler has performed the technical procedures and has assisted with the computations.

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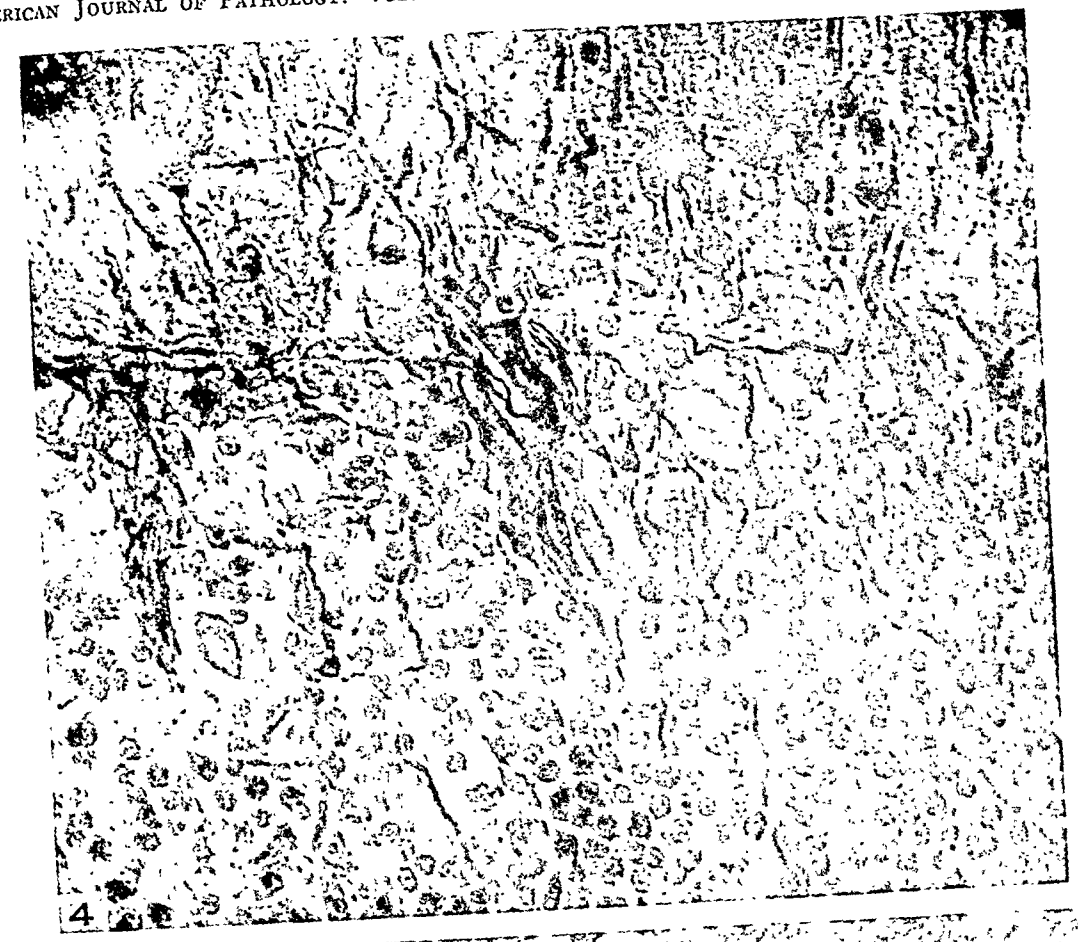
DESCRIPTION OF PLATES

PLATE 165

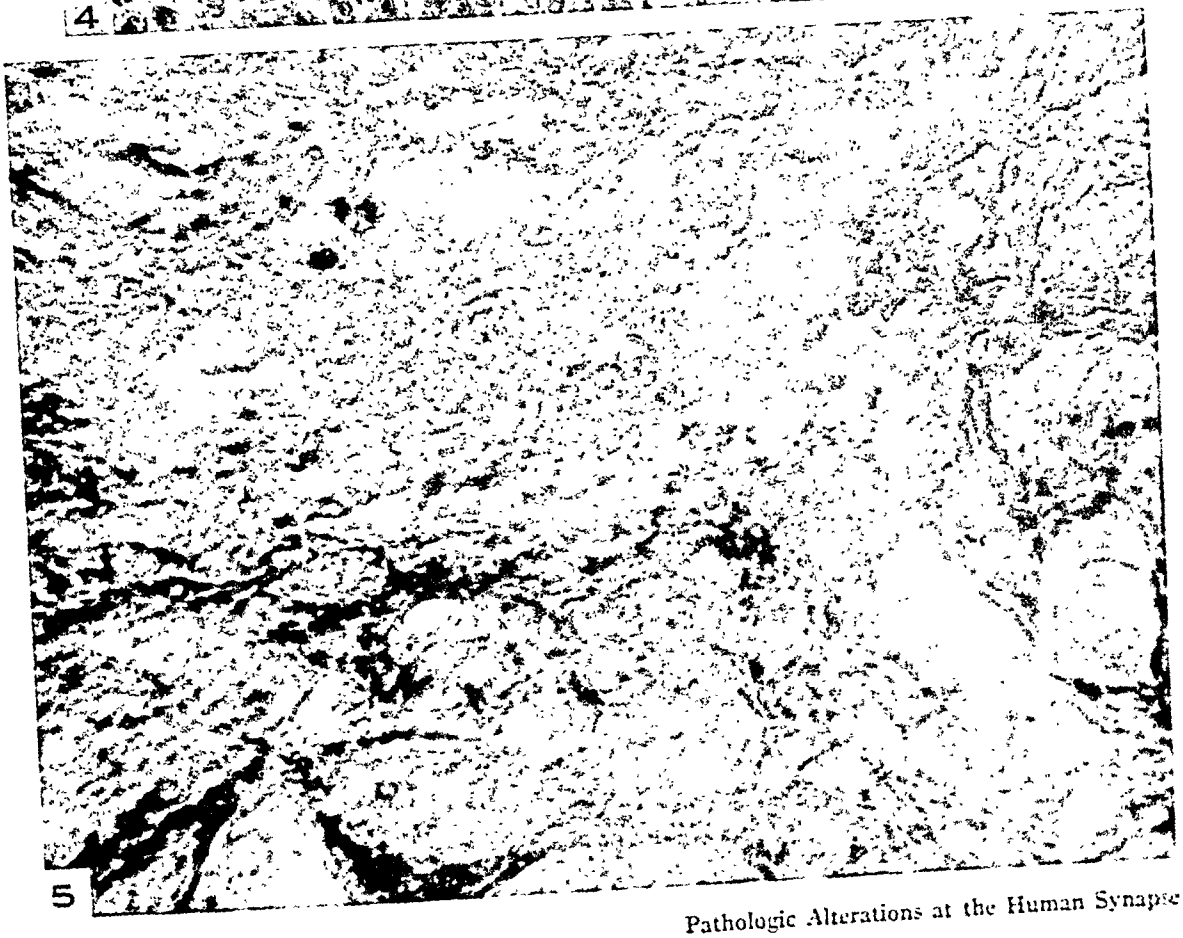
FIGS. 1–3. Photomicrographs of illustrative groups of boutons to which the schema of the morphologic classification as "types" and "forms" refers. Small loops are designated S; large loops, L; filamented loops, F; fibrillated bulbs, B, and opaque and granular masses, M. Thickened forms are marked prime (′) and granular forms, double-prime (″). Gn (Fig. 3) indicates a granular neurofibril. There are different degrees of granulation effect in the three figures. Figures 1 and 2, × 1400; Figure 3, × 1800.

Distribution of Forms

	Regular	Thickened	Granular
Type 1. Small loops	Fig. 2 S	Fig. 1 S′	Fig. 1 S″
Type 2. Large loops	Fig. 1 L	Fig. 1 L′	Fig. 3 L″
Type 3. Filamented loops	none shown	none shown	Fig. 2 F″
Type 4. Fibrillated bulbs	Fig. : B	Fig. 1 B′	Figs. 2, 3 B″
Type 5. Opaque and granular masses		Fig. 1 M′	Fig. 3 M″



4



5

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PLATE 166

FIG. 4. Photomicrograph showing persistent basket terminals following degeneration of Purkinje cells. × 600.

FIG. 5. Photomicrograph showing persistent boutons on an anterior horn cell undergoing degeneration by neuronophagia. × 1200.

HYPERPLASIA OF MEGAKARYOCYTES IN PNEUMONIA AND ITS RELATIONSHIP TO LEUKOBLASTIC HYPERPLASIA OF THE BONE MARROW *

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In 1908 Dickson [1] stated that the megakaryocytes are, on the whole, somewhat increased in number and size in pneumonia and septicemia, and are less frequently unaltered or diminished. These observations were based on the inspection of the upper part of the shaft of the femur and of the ribs. Frey [2] counted the bone marrow giant cells at the junction of the lower and middle thirds of the femur in 50 cases of widely varying diseases, 10 of which were acute infections. The highest count occurred in a case of polycythemia vera and the next highest in one of pneumonia. He stated that the increase of megakaryocytes in pneumonia is a typical example of the changes of these cells in acute infectious diseases. The findings in his cases of acute infections are difficult to interpret because of lack of suitable controls and because of the bone examined. Nickerson and Sunderland [3] counted the number of megakaryocytes per unit area of vertebral marrow in 3 cases of idiopathic thrombocytopenic purpura, 5 of secondary thrombocytopenic and symptomatic purpura, 2 of unclassified purpura and 8 of sudden death due to varying causes. In this series of 18 cases the highest megakaryocytic count was found in 1 case of idiopathic thrombocytopenic purpura and the next highest count was in one of the cases of secondary thrombocytopenic and symptomatic purpura with pneumonia. Rosenthal [4] stated that in infections, especially pneumonia, there is a marked stimulation and increased formation of megakaryocytes, occurring simultaneously with stimulation and increase of leukopoietic tissue.

Except where stated the above reports were unaccompanied by factual evidence.

MATERIALS AND METHODS

The material was derived from 306 human adults, unselected except for the exclusion of primary hemopoietic diseases. In 39 of these 306 cases, pneumonia of some type had previously been listed in the anatomical diagnosis as the primary disease. This independent consideration of these cases as primary pneumonia permitted an unbiased selection. Only cases of well developed pneumonia were studied, those of terminal bronchopneumonia not being a part of this series. For

* Received for publication, February 6, 1942.

cytes into A, B and C forms is an extremely useful one. Myelocyte C has an oval to slightly indented nucleus. The cytoplasm shows no basophilia and is packed with specific granules. Myelocyte A has less than ten specific granules in its cytoplasm, which is a gray-blue in color. Also included in the myelocyte A group is a cell which represents a transition stage between myeloblasts and myelocytes with only a few specific granules. This cell has a gray-blue cytoplasm which lacks granules. It differs from the myeloblast in having a more abundant cytoplasm and in lacking the intense basophilia of the cytoplasm. This type of myelocyte A is pictured as cell number 15 in Figure 67 on page 82 in Maximow and Bloom's [9] Textbook of Histology. Myelocyte B represents a stage of maturation intermediate between A and C.

Selection of the sites for study of the marrow depends on knowledge of the normal and pathological distribution of the red and fatty marrow in the different bones. According to Neumann [10] (1882), in adults active red marrow is found almost without exception in the skull, vertebrae, sternum, ribs and other bones of the trunk. He stated that in healthy adult persons either all the small and large bones of the extremities contain exclusively fatty marrow or the active red marrow is limited to the upper part of the humerus and femur, that is, the upper epiphysis and a smaller or larger segment of the adjacent diaphysis. There are various transition stages between these two pictures. Piney [11] agreed with Neumann as to the normal state of the marrow of the vertebrae, sternum and ribs in the adult except that he added that by the age of 25 years the marrow of the ribs adjacent to the cartilages for a distance of about 1 inch is fatty. He also agreed essentially with Neumann's findings with respect to the distribution of the red and fatty marrow in the shafts of the long bones in normal adults, stating that in extremely advanced age the diaphyseal patch of red marrow at the upper end of the humerus and femur may be almost completely replaced by fatty marrow. Piney further reported that the shaft of the humerus in adults appears to contain a larger quantity of active marrow than that of the femur. Schmorl [12] emphasized the constancy of hemopoiesis in vertebral marrow as compared to that of all other bones.

Neumann [10] stated that the pathological transformation of fatty marrow into red marrow in the long bones takes place in a centrifugal direction with respect to the trunk. It successively extends not only over the whole humerus and femur but also to the upper epiphysis, diaphysis and lower epiphysis of the bones of the forearm, tibia and fibula, and later to the bones of the hands and feet. He added that the red marrow in the upper extremities appears to extend farther

controls a series of 16 cases, those of patients dying of traumatic injuries within 12 hours after injury, was used. Also the clinical records of all cases were surveyed for pertinent data.

Bone marrow from a lumbar vertebra and the upper third of the diaphysis of the humerus was available for study in the 306 cases. In addition to the usual bony sections from the vertebra, vertebral pulp was studied. The latter was obtained by sawing away the cortex of the vertebra and driving a chisel into the spongy bone. The marrow pulp which exuded around the chisel was placed on a small square of filter paper and was then carried through the various technical processes similar to curettings in general.[1] Thus the cytology of nondecalcified vertebral marrow could be studied.

All marrow was fixed in Zenker's-formaldehyde solution, embedded in paraffin and stained with hematoxylin and eosin and with azure II and eosin.[5] Thin slices of spongy bone of the vertebra, cut with a jeweler's saw, were decalcified not over 48 hours in equal parts of an 85 per cent aqueous solution of formic acid and a 20 per cent solution of sodium citrate.[5]

For the purpose of making differential counts of megakaryocytes, sections cut from the paraffin-embedded marrow of the humerus and vertebral pulp were handled according to Wright's technic.[6] In order to control this modified technic, blocks of fresh femoral marrow from rabbits were treated strictly according to Wright's method except that the fixing fluid as altered by Downey [7] was used. Blocks of rabbit's marrow were also treated similarly to the human marrow. By comparing the sections of rabbit's marrow obtained by the two methods with the sections of human marrow, it could be determined whether the latter were technically satisfactory. Figure 1 shows an adult megakaryocyte of the rabbit stained strictly according to Wright's technic after Downey's fixation, and Figure 2 shows an adult human megakaryocyte treated according to the modified Wright's technic herein described.

The terminology in general is that used by Sabin and Miller.[8] Certain explanations are necessary for its adaptation to the material in this study and to the sections stained with azure II and eosin. The primitive cell of this classification is a small round cell scattered throughout the marrow. It differs from the small lymphocyte in having a lower content of chromatin in the nucleus and in lacking rod-shaped mitochondria in the cytoplasm. With the technic and material used here, I was unable, on the basis of cytology alone, to differentiate this cell from the small lymphocyte. Therefore I have called all such cells the small round cells of the bone marrow. The classification of the myelo-

humerus and the junction of the lower and middle thirds of the femur and tibia. Since the distribution of red marrow differs in an orderly manner, not only in different bones but also in the same bone, care should be taken to examine the marrow at definite locations and proper controls should be available for these particular locations.

Preliminary Findings

By inspection of sections of vertebral marrow from the 306 unselected cases and comparison with controls without any other knowledge of the cases, the presence or absence of an increase of megakaryocytes was determined. Twenty-six cases showed hyperplasia of these cells. The increase was variable throughout the different parts of the same section with a tendency to be marked in focal areas of the marrow, and varied in degree from case to case (Figs. 3 and 4). The vast majority of the megakaryocytes were adult and degenerate with complicated and polymorphous nuclei. Young megakaryocytes were rare. Bizarre mitotic figures [9,19] (Fig. 5) of the megakaryocytes were found in 12 cases of pneumonia and were not searched for in the cases without pneumonia. Seven of these 12 showed hyperplasia of megakaryocytes and 5 did not. No spindles were seen. There was never any evidence of cytoplasmic division associated with these mitotic figures.

Of the 26 cases showing an increase in megakaryocytes, 17, or 65 per cent, were cases of primary pneumonia. Of the 306 unselected cases, only 39, or 12 per cent, were cases of primary pneumonia. This is a significant difference and demonstrates a definite relationship between pneumonia and hyperplasia of megakaryocytes. The 9 cases of hyperplasia of megakaryocytes without primary pneumonia, together with pertinent data, are listed in Table I.

The Control Series

The number of megakaryocytes in the bone marrow was based on the enumeration of these cells in the bony sections of vertebral marrow. The number was much more constant here than in the marrow of the humerus. For example, the marrow of the humerus of case 12 of the controls (Table II) was completely fatty (Fig. 6) and showed no megakaryocytes. That from the humerus of case 1 (Table II) showed the maximum degree of hemopoietic cellularity of the controls for this location (Fig. 7). A considerable number of megakaryocytes were present. Obviously what is normal for the humeral marrow of case 1 would be grossly abnormal for that of case 12. There were all variations between these two examples. The difficulty in detecting hyperplasia of the megakaryocytes in the humeral marrow is apparent because of such wide variations in the normal.

downward than in the lower extremities. With respect to the diaphysis of the humerus, femur and tibia, I interpret these statements of Neumann in the following manner. The extension of red marrow in the humerus is greater than in the femur and hyperplasia in the femur definitely precedes that in the tibia. Also the hyperplasia begins in the upper end of each bone.

Seecof [13] advanced the concept that hyperplasia does not take place uniformly in the long bones. It occurs first in the humerus, then in the femur and last in the tibia. Later I [14] found, in the examination of the marrow at the junction of the lower and middle thirds of the humerus, femur and tibia in 100 unselected cases, that the presence or absence of hyperplasia, as judged by the sample taken, occurred in certain orderly combinations. Hyperplasia may be (1) absent in all three bones, (2) present in the humerus alone, (3) present in humerus and femur and absent in tibia, or (4) present in all three bones. These data adequately support Seecof's concept. Strumia [15] and Custer and Ahlfeldt [16] noted the tibial marrow to be less active than that of the femur. Seecof,[17] in certain cases of pernicious anemia studied before 1924, found the marrow of the long bones aplastic and the flat bones hyperplastic. This emphasizes the fallacy of examining only the marrow of the long bones. Custer and Ahlfeldt recommended inclusion of the vertebral marrow among the sites to be studied. According to Wienbeck [18] it is necessary to examine the vertebrae in order to interpret the state of the bone marrow.

As the data which follow in this report will show, changes are best detected by a study of the vertebral marrow as compared to that of the upper end of the femur or humerus. This is true because in any individual case the structure of the marrow previous to the onset of disease is best judged in the vertebrae. Therefore, pathological changes can be best detected here. Also cases with hyperplasia in the vertebrae and none in the long bones would be missed by examination of the latter only. The study of the marrow of the vertebrae has certain obvious technical advantages over that of the other flat bones at autopsy.

The above review shows that the examination of the marrow of the humerus has the advantage over that of other long bones of revealing the earliest evidence of hyperplasia in the long bones. Examination of the upper end should reveal the earliest change in the marrow of a particular tubular bone.

For an extensive survey, Custer and Ahlfeldt [16] recommended the study of the marrow of the vertebrae, sternum, rib, femur and tibia. For the same purpose I have studied the marrow of the lumbar vertebrae, the body of the sternum, the upper third of the shaft of the

The results, together with other appropriate data, are listed in Table II. The megakaryocytes in the controls varied from 77 to 208 per 100 microscopical fields, or 1791 to 4837 per cmm. of marrow.

In order to determine the cellular pattern of the megakaryocytes, differential counts were done, each based on 200 of these cells. The marrows were prepared according to the modified Wright technic. Only those considered to be satisfactory from a technical standpoint were used. The preparations from 5 of the 16 control cases and from 2 other additional patients, dying of traumatic injuries within 12 hours

TABLE II
Data on Control Series

Number of case	Sex	Age	Duration	Diagnosis	Megakaryocytes per 100 microscopical fields	Megakaryocytes per cmm of marrow
1	F	55	1 hour	Traumatic injuries	112	2605
2	F	Adult	3 hours	Traumatic injuries	140	3256
3	M	55	1 hour	Traumatic injuries	77	1791
4	M	51	2 hours	Traumatic injuries	146	3326
5	M	33	6 hours	Traumatic injuries	121	2814
6	F	Adult	1 hour	Traumatic injuries	92	2140
7	F	70	1 hour	Traumatic injuries	91	2116
8	M	73	12 hours	Traumatic injuries	93	2162
9	F	42	?	Traumatic injuries	136	3160
10	M	19	3 hours	Traumatic injuries	208	4837
11	M	Adult	3 hours	Traumatic injuries	131	3047
12	M	33	10 minutes	Traumatic injuries	81	1884
13	F	67	6 hours	Traumatic injuries	173	4023
14	M	65	3 hours	Traumatic injuries	126	2930
15	M	Adult	1 hour	Traumatic injuries	78	1814
16	M	60	2 hours	Traumatic injuries	88	2046

of injury, were satisfactory. These included 3 of vertebral pulp and 4 of active humeral marrow. The classification used was similar to that of Custer and Krumbhaar [21] and Nickerson and Sunderland,[3] the exact terminology of the latter being employed. These authors divided the megakaryocytes into three types: (1) young megakaryocytes; (2) adult megakaryocytes; (3) degenerate megakaryocytes. The young form is a large, moderately basophilic cell with a nucleus that is oval or shows early nuclear polymorphism. The nucleus has a characteristically arranged heavy chromatin network. Its cytoplasm is relatively scanty in amount and granularity is absent. The adult megakaryocyte presents a complicated, more or less vesicular nucleus with abundant cytoplasm. It is filled with fine reddish violet to reddish blue granules.[19, 22] A hyaline marginal zone may or may not be present. Pseudopods are frequent. The cytoplasmic margins are at times frayed. The degenerate cell is characterized by a shrunken and faded or pyknotic nucleus. The cytoplasm stains poorly and granularity is absent. Typical megakaryocytic nuclei without cytoplasm, or naked nuclei, were

The megakaryocytes were counted per unit area of marrow [20] of the vertebrae and 100 microscopical fields [2] were chosen as the unit in which to count them. Trabeculae of bone were excluded from this area and the more cellular regions were examined. The optical system

TABLE I

Data on Nine Cases of Hyperplasia of Megakaryocytes Without Pneumonia

Number of case	Age	Sex	Diagnosis	Leukoblastic hyperplasia
1	40	M	Recent cholecystectomy for chronic cholecystitis and cholelithiasis; chronic suppurative peritonitis, localized; pulmonary embolus	Present
2	77	M	Diabetes mellitus; carbuncle of neck; pyemia; bronchopneumonia; cardiac hypertrophy and dilatation	Present
3	52	M	Subacute glomerulonephritis; anasarca; hyperplastic lymphadenitis; chronic splenitis; splenomegaly (540 gm.); cerebral hemorrhage; luetic aortitis and valvulitis; chronic passive hyperemia of liver; pulmonary edema; (unclassified hyperplasia of bone marrow; anemia; red blood cells 2.5 million)	Absent
4	44	F	Congenital polycystic kidneys; uremia; chronic suppurative pyelonephritis	Present
5	30	M	Amyloidosis of kidneys; uremia; fibrinous pericarditis; chronic endocarditis; mitral stenosis and insufficiency; cardiac hypertrophy, right ventricle; vegetative endocarditis of mitral valve	Present
6	70	F	Diabetes mellitus; chronic suppurative cystitis; acute suppurative pelvic peritonitis	Present
7	42	F	Recent panhysterectomy and appendectomy for chronic salpingo-oophoritis; generalized peritonitis	Present
8	63	M	Sclerosis of coronary arteries; myocardial infarction, recent; fibrinous pericarditis; cardiac hypertrophy	Present
9	75	M	Carcinoma of the pancreas; sclerosis of coronary arteries; healed myocardial infarction with mural thrombus; cardiac hypertrophy; hyperplasia of prostate gland; dilatation of ureters and pelves; renal calculus; diabetes mellitus; gangrene of right toe; cholelithiasis; (postmortem blood culture, *Streptococcus hemolyticus*)	Present

used was a Zeiss objective 40 with a numerical aperture of 0.65 and 10x eyepiece. From the number of megakaryocytes per 100 microscopical fields the number per cubic millimeter of vertebral marrow was calculated.*

* Since the volume of a cylinder equals $\Pi r^2 h$, if r is the radius of the microscopic field, measured with a stage micrometer, and h the thickness of the section, which was approximately 5 μ, the volume of 100 such cylinders equals $0.165^2 \times 0.005\Pi \times 100$ or 0.043 cmm. Then the megakaryocytes per cubic millimeter of marrow equal:

$$\frac{\text{number of megakaryocytes in 100 fields}}{0.043}.$$

These calculated results are admittedly an arbitrary and not an absolute value of the number of megakaryocytes per cubic millimeter of marrow. Nevertheless, they permit comparison with the results of others using the same method and the marrow of the lumbar vertebra.

quite well defined at low magnification, appearing as closely grouped, small, dark nuclei (Figs. 8 and 9).

As previously described, the marrow of the upper third of the humerus, in contrast to that of the lumbar vertebrae, showed an extreme degree of variation in hemopoietic cellularity (Figs. 6 and 7). The size and number of islands of erythropoiesis were likewise quite variable in the active humeral marrows. They varied from less than 1 per high-power field (as in case 15, Table II; Fig. 11) to numerous islands per high-power field (as in case 16, Table II; Fig. 12). There were various intermediate grades of erythropoiesis.

The cellular pattern or qualitative type of hemopoiesis both in the vertebral and active humeral marrows, in contrast to the varying degree of cellularity, was quite constant. The predominant cells of erythropoiesis were the normoblast and the erythroblast in a late stage of maturation. Earlier forms of erythroblasts were infrequent. Myeloblasts were likewise rare but were occasionally found widely scattered. Small round cells and plasmalike cells, scattered widely and occasionally occurring in groups of two or three, were present. Lymphoid nodules were observed. These have been discussed in a previous report.[23] Granulopoiesis was made up almost exclusively of neutrophilic myelocytes C (Fig. 13). Young neutrophilic myelocytes, or myelocytes A and B, which have varying degrees of basophilia and varying numbers of granules in the cytoplasm according to their maturity, were not a prominent feature of the picture. Up to 30 eosinophils per oil immersion field (Zeiss objective 90 and 7x eyepiece) were noted. Metamyelocytes and polymorphonuclear leukocytes were infrequent in this postmortem bone marrow. Rare and widely scattered basophilic myelocytes were present.

Cases of Pneumonia

Megakaryocytes in Pneumonia

The megakaryocytes per 100 microscopical fields were counted and the number per cubic millimeter calculated, as in the control cases. The data are recorded in Table IV. Of the 39 cases of pneumonia, 19, or 49 per cent, showed an increase of megakaryocytes, that is, above 5,000 per cc. of vertebral marrow. Four showed a value below normal and the remainder fell within the range of the controls.

It is appropriate at this point to evaluate the accuracy of the diagnosis of hyperplasia of megakaryocytes by inspection of the vertebral marrow as done in the preliminary data. In Table IV are listed these results as determined by inspection in the 39 cases of pneumonia. They agreed with those obtained by actual counting of the megakaryo-

included in the degenerate group. Those cells with well preserved cytoplasm with granules, but with some evidence of nuclear degeneration, were arbitrarily classified as adult megakaryocytes.

The results are tabulated in Table III. In the 7 control cases the cellular pattern of the megakaryocytes did not vary markedly. The young cells constituted 3 per cent and less, the adult ones 71 to 85 per cent and the degenerate ones 13 to 28 per cent of the number of megakaryocytes present.

TABLE III

Cellular Pattern of the Megakaryocytes of the Control Cases

Number of case	Bone marrow	Megakaryocytes			Unclassified
		Young	Adult	Degenerate	
		(%)	(%)	(%)	(%)
7	Vertebral	0	78	20	2
9	Humeral	0	71	28	1
10	Humeral	1	85	13	1
13	Vertebral	2	74	23	1
14	Humeral	3	72	23	2
	Humeral	0	72	27	1
	Vertebral	0	72	28	0

Because of the results to follow in the cases of pneumonia, it is necessary to emphasize certain histological and cytological findings of the marrow of the control series. In the vertebral marrow there was considerable variation in the relative area of tissue occupied by hemopoietic cells and fat cells. Those of the 16 control cases showed a variation ranging from one-fourth or one-third of the area of the sections to be occupied by hemopoietic cells (case 3, Table II; Fig. 8) to three-fourths of the area to be occupied by hemopoietic cells (case 10, Table II; Fig. 9). The entire range of variation was present not only in the sections of marrow from the different cases but in different parts of the same section from certain cases. Not infrequently small isolated fatty foci (Fig. 10) were found in an otherwise active vertebral marrow of the above described limits. It is to be noted that a degree of cellularity which would be normal for case 10 would be gross hyperplasia for case 3. The difficulty of detecting moderate hyperplasia in individuals, such as in case 3, on the basis of degree of cellularity alone is apparent. This brings out the need for a diagnostic criterion of hyperplasia in addition to that of increased hemopoietic cellularity.

An outstanding histological landmark of the vertebral marrow from all 16 control cases was the presence of well defined islands of closely packed erythropoietic cells. These islands averaged 3 to 8 in number per high-power field (Zeiss objective 40 and 7x eyepiece). They were

cytes in 35, or 90 per cent, of the cases. Thus it is seen that the diagnosis of hyperplasia of megakaryocytes by inspection of the vertebral marrow and comparison with adequate controls is a reasonably accurate method in any series of cases. This obviously is a great advantage since it allows for the analysis of a large number of cases in a relatively short time as compared to the more laborious and time-consuming method of enumeration.

The cellular pattern of the megakaryocytes was determined by differential counts of the three types of cells, as in the control cases. Thirteen sections of marrow, including 5 of vertebral pulp and 8 of active humeral marrow, from 12 of the 39 cases of pneumonia were considered technically satisfactory. They were prepared according to the modified Wright technic. Ten of these sections showed a hyperplasia of the megakaryocytes and 3 did not. These differential counts did not vary significantly from those of the controls. The adult megakaryocytes with their complicated nucleus and finely granular cytoplasm constituted 68 to 84 per cent of all these giant cells.

The occurrence of a thrombocytopenia during the febrile period followed by a thrombocytosis during convalescence in acute infectious diseases, including pneumonia, is well known.[24-27] In these cases of pneumonia the presence of normal or increased numbers of megakaryocytes in all except 4 cases, together with a normal cellular pattern of these cells, is evidence that the thrombocytopenia does not depend on changes in the megakaryocytes.

Parallelism of Megakaryocytic and Leukoblastic Hyperplasia

Associated with the increase of megakaryocytes there were certain other changes in the vertebral marrow. These consisted of a marked increase in hemopoietic cellularity with a corresponding decrease in fat cells. This was accompanied by a marked decrease to almost complete absence of well defined, compact islands of erythropoiesis (case 31, Table IV; Fig. 3). Even a lesser degree of cellularity at about the upper limits of normal was always accompanied by a decrease in the size and number of islands of erythropoiesis and in certain cases by almost complete absence of such islands (case 22, Table IV; Fig. 4). Neutrophilic myelocytes in varying stages of maturation, together with fewer metamyelocytes and polymorphonuclear leukocytes, were the predominant cells of granulopoiesis.

The degrees of maturity in granulopoiesis could be divided into four groups for descriptive purposes. No sharp line of demarcation existed between the different groups. In group I there was no variation from the controls (Fig. 13). Group II showed myelocytes C still to be

TABLE IV

Data on Cases of Pneumonia

	Number of case	Megakaryocytes in 100 microscopical fields*	Megakaryocytes per cmm. of vertebral marrow†	Megakaryocytic hyperplasia by inspection of vertebral marrow	Leukoblastic hyperplasia of vertebral marrow	Cellular pattern of granulopoiesis; group	Evaluation of humeral marrow	Remarks
A. Cases showing no increase of megakaryocytes	1	63	1465	Absent	Doubtful		Active	None
	2	122	2837	Absent	Absent		Fatty	None
	3	161	3744	Absent	Absent		Active	Shift to left in erythropoiesis in vertebra; no anemia
	4	207	4814	Absent	Absent		Fatty	None
	5	202	4698	Absent	Absent	IV	Active	None
	6	128	2977	Absent	Absent	III	Active	None
	7	123	2860	Absent	Absent		Fatty	None
	8	132	3070	Absent	Absent	III	Active	None
	9	51	1186	Absent	Absent	II	Active	None
	10	39	907	Absent	Absent	III	Active	Unclassified hyperplasia in vertebra; anemia; RBC 1.62 million
	11	132	3069	Absent	Absent	II	Active	None
	12	122	2837	Absent	Absent	I	Active	Cellular pattern of granulopoiesis in humerus, group II
	13	207	4814	Absent	Absent		Fatty	None
	14	93	2163	Absent	Absent	II	Active	None
	15	162	3767	Absent	Present	III	Meg. hyp.‡ and hyp.	None
	16	98	2279	Absent	Absent	I	Hyp.	Unclassified hyperplasia in vertebra; anemia; RBC 2.6 million
	17	137	3186	Absent	Doubtful	III	Active	None
	18	61	1419	Absent	Absent	II	Active	None
	19	90	2093	Absent	Absent		Active	None
	20	201	4674	Present	Present	II	Active	None
B. Cases showing an increase of megakaryocytes	21	289	6721	Present	Present	II	Active	None
	22	305	7093	Present	Present		Fatty	None
	23	238	5539	Present	Present		Fatty	None
	24	241	5605	Present	Present	I	Meg. hyp.	None
	25	250	5814	Present	Present	III	Meg. hyp.	None
	26	347	8069	Present	Present	III	Meg. hyp. and hyp.	None
	27	403	9372	Present	Present	IV	Meg. hyp.	None
	28	249	5790	Absent	Present	III	Meg. hyp. and hyp.	None
	29	227	5279	Present	Present	II	Meg. hyp.	None
	30	263	6116	Absent	Present		Active	None
	31	575	13372	Present	Present	IV	Meg. hyp. and hyp.	None
	32	322	7484	Present	Absent	II	No section	None
	33	342	7953	Present	Present	IV	Fatty	None
	34	265	6162	Absent	Present	III	Meg. hyp. and hyp.	None
	35	263	6116	Present	Present	II	Active	None
	36	220	5116	Present	Present	I	Active	Cellular pattern of granulopoiesis in humerus, group I
	37	229	5326	Present	Present	IV	Active	None
	38	429	9976	Present	Present	I	Fatty	None
	39	337	7837	Present	Present	III	Fatty	None

* Normal, 77 to 208.

† Normal, 1791 to 4837.

‡ Hyp. = hyperplasia; Meg. = megakaryocytic; Active = degree of hemopoietic cellularity within

at the upper limits of the controls. However, the change from normal cellularity in this case was shown by the almost complete absence of islands of erythropoiesis. I interpret such a case as having a degree of cellularity, prior to the onset of pneumonia, at the lower limit of normal, similar to that of case 3 of the controls, Figure 8. With the occurrence of the disease there has been an increase of leukoblastic cells accompanied by a decrease in the size and number of erythropoietic islands. It was only through the latter change that an increase of leukoblastic cells could be detected. In 9 of the cases of pneumonia the erythropoietic component of the criteria of leukoblastic hyperplasia was the deciding factor in making a positive diagnosis.

Using the above criteria of leukoblastic hyperplasia, the results obtained are tabulated in Table IV. In 34, or 87 per cent, of the cases of pneumonia there was an exact parallelism between megakaryocytic hyperplasia and leukoblastic hyperplasia. In the 20 cases without an increase of megakaryocytes there was also an absence of leukoblastic hyperplasia in 16. Of the 4 exceptions, the lack of parallelism in cases 1 and 17 was doubtful (Table IV). On the other hand, in the 19 cases with hyperplasia of megakaryocytes there was also a leukoblastic hyperplasia in 18. In such an organ as the bone marrow where changes may be very difficult to detect and evaluate, this is a high degree of correlation.

As seen in Table I, 8 of the 9 cases of hyperplasia of megakaryocytes without primary pneumonia showed both leukoblastic and megakaryocytic hyperplasia. In at least 6 of them there was a bacterial infection (Table I). In only 1 of these was pneumonia present in addition, and it was a terminal bronchopneumonia. Thus this hyperplasia of megakaryocytes and leukoblastic cells must be regarded as a type reaction which occurs frequently in primary pneumonia and at least in certain other infectious diseases.

Evaluation of the Humeral Marrow

As pointed out in the control series, the marrow of the upper third of the humerus normally shows a wide range of variability as to both the degree of cellularity and the number and size of the islands of erythropoiesis. This makes it difficult to determine what the picture of this marrow may be before the onset of any particular disease. Only those changes clearly and definitely beyond the wide range of the normal degree of cellularity can be recognized.

The marrow of the humerus in the pneumonia series could be classified into five types in the following manner. (1) Fatty. Examples of this group showed no hemopoietic cellularity. (2) Active. In these

the predominant cells and there was a slight but definite increase in myelocytes A and B. This group represented a slight shift to the left in granulopoiesis. In group III there was a moderate decrease in myelocytes C and a moderate increase in myelocytes A and B. Myeloblasts were more frequent than usual. Group IV represented a marked shift to the left in granulopoiesis. Myelocytes C were almost completely absent and myelocytes A were the predominant cells (case 33, Table IV; Fig. 14). Also myeloblasts and myelocytes B were increased. Frequently in all groups there was an increase of the small round cells of the bone marrow, which were scattered throughout, and an increase of the plasma cell-like cells. The latter were frequently collected into groups of four or five cells. Neutrophilic metamyelocytes, leukocytes and eosinophils showed no particular change. Differences between the cellular pattern of the vertebral and of the active humeral marrow of the same case were only slight. For example, granulocytopoiesis in the humerus might be classified as group IV while that in the vertebra as group III. In 29 of the 39 cases the marrow preparations were considered satisfactory for this cytological study. The results of the classification of granulopoiesis in the vertebra into the four groups are listed in Table IV. It is seen that these changes in the cellular pattern of granulopoiesis occurred irrespective of the presence or absence of an increase in megakaryocytes or leukoblastic cells in general.

In addition, 2 cases (nos. 10 and 16, Table IV) showed what might be considered as hyperplasia of both erythroblastic and leukoblastic cells. Both of these cases had a severe unexplained anemia. One other case (no. 3) showed a moderate shift to the left in erythropoiesis. This was a typical case of primary pneumonia of 13 days' duration due to type III pneumococcus. There was no anemia. Other than this single case there was no detectable qualitative change in erythropoiesis.

To emphasize the changes in the vertebral marrow associated with an increase in megakaryocytes, leukoblastic hyperplasia may be defined as follows. Leukoblastic hyperplasia is characterized by a degree of hemopoietic cellularity at or above the upper limits of normal and the neutrophilic myelocyte in various stages of maturation is the predominant cell of granulopoiesis. This is accompanied by a change in erythropoiesis varying from a decrease in the size and number of erythropoietic islands to almost complete absence of such.

It is emphasized that in addition to the well recognized criterion of increased cellularity the decrease in size and in number of islands of erythropoiesis is a valuable aid in detecting this leukoblastic hyperplasia. For example, Figure 4 illustrating the vertebral marrow of case 22, Table IV, showed a degree of hemopoietic cellularity that is

granulopoiesis. There was no essential difference in this respect between the marrows of the two bones. The humeral marrow was active in all 7 of these cases. There were no studies of the cellular pattern in the 6 remaining cases.

Group B. In 6 cases (nos. 22, 23, 33, 36, 38 and 39) there was a leukoblastic and megakaryocytic hyperplasia in the marrow of the vertebra while that of the humerus was entirely normal. In 5 the humeral marrow was fatty. The cellular pattern was normal in 2 cases, one of which had a fatty and the other a normal active humeral marrow. Granulopoiesis was shifted to the left in 2 and not studied in 2 cases.

Group C. Five cases (nos. 20, 21, 30, 35 and 37) showed a leukoblastic hyperplasia and 4 of these had an increase in the megakaryocytes in the vertebrae. These changes were not demonstrable in the associated humeral marrows, all of which were active. There was a shift to the left in granulopoiesis of both bones in the 4 cases so studied.

Group D. In 8 cases (nos. 24, 25, 26, 27, 28, 29, 31 and 34) there was a leukoblastic and megakaryocytic hyperplasia in the vertebra and a hyperplasia of the marrow or increase in megakaryocytes, or both, in the humerus. A shift to the left in granulopoiesis was present in 7 cases and absent in 1 case. There was agreement in this respect between the marrows of the two bones in the same individual.

The presence of leukoblastic and megakaryocytic hyperplasia in the vertebra associated with a normal humeral marrow in the 6 cases of group B, plus the presence of such hyperplasia in the marrow of both bones in the 8 cases of group D, permits the concept that these changes begin first in the vertebrae and only later take place in the diaphysis of the humerus. The cases of groups A and C are in agreement with this concept.

The data on the qualitative changes show that when there is a shift to the left in granulopoiesis the changes in the two bones are similar provided that there is activity of the humeral marrow. If the humeral marrow is normally fatty in any individual case, the shift to the left would begin in the vertebrae since there would be no hemopoiesis in the diaphysis of the humerus at first. This possibility is exemplified by the 5 cases of group B. However, the constant finding of similar qualitative changes in granulopoiesis in both bones of the same individual when the humeral marrow was active indicates that red marrow, wherever present, functions in this respect at or about the same time. This does not deny the concept that an increase above the normal level in megakaryocytes and leukoblastic cells in general takes place first in the vertebrae and later in the humerus.

marrows there was active hemopoiesis with the same variable extent and degree of hemopoietic cellularity and erythropoiesis as in the controls. They occurred with and without qualitative changes in granulopoiesis. (3) Megakaryocytic hyperplasia. Here the megakaryocytes were markedly increased focally or diffusely throughout the marrow (case 25, Table IV; Fig. 15). The remainder of the marrow was as in (2). (4) Hyperplasia of the marrow. This marrow showed an increase of hemopoietic cellularity beyond the normal range with no demonstrable increase of megakaryocytes. Only one case fell in this group. (5) This last group consisted of those showing both general hyperplasia and megakaryocytic hyperplasia (case 31, Table IV; Fig. 16). All of these cases showed some degree of shift to the left in granulopoiesis. The islands of erythropoiesis varied from few in number and minute in size to moderate in size and number. The results of this classification are tabulated in Table IV.

Of the 38 cases of pneumonia in which the humeral marrow was studied, 9 showed an increase of megakaryocytes. In only 5 of these was there likewise a demonstrable increase in the degree of hemopoietic cellularity. One would hardly be justified, on the basis of such data from the marrow of the humerus, in concluding that a parallelism exists between megakaryocytic and leukoblastic hyperplasia. In addition to the above 9 cases, study of the vertebral marrow by the comparative method of inspection showed an increase of megakaryocytes in 10 other cases. Study of the humeral marrow alone would have missed these 10 cases. In only 3 cases was an increase of megakaryocytes diagnosed in the humeral and not in the vertebral marrow by inspection. The necessity of studying the vertebral marrow in detecting and interpreting reactions is even more strikingly illustrated by the findings relative to leukoblastic hyperplasia as previously defined. Leukoblastic hyperplasia was demonstrated by the study of the vertebral marrow in 20 cases while hyperplasia in the humerus, as defined, was present in only 6 cases.

For the sake of analysis of the findings in the humeral and vertebral marrows of the same individual, as recorded in Table IV, the cases of the pneumonia series may be divided into four groups, A, B, C and D. The data below were based on the enumeration of the megakaryocytes in the vertebral marrow. Three cases (nos. 1, 17 and 32) were excluded because of insufficient or doubtful findings.

Group A. In 13 cases (nos. 2, 3, 4, 5, 6, 7, 8, 9, 11, 13, 14, 18 and 19) megakaryocytic and leukoblastic hyperplasia were absent in both the marrow of the vertebra and of the humerus. Cytological studies on 7 of this group showed a greater or lesser degree of shift to the left in

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Of the remaining cases not included in this summary, 2 (nos. 12 and 15) are possible but not conclusive exceptions to the concept (Table IV). The other 2 cases (nos. 10 and 16) with their unclassified hyperplasia are not exceptions to the rule.

Miscellaneous

The material for this report was collected prior to the advent of treatment of pneumonia with the sulfonamide drugs. Two cases only (nos. 13 and 32) were treated with prontylin. Antipneumococcus serum was administered to 9 of the 39 cases. Seven of these showed hyperplasia of megakaryocytes. However, 12 cases which had received no serum therapy showed an increase of the megakaryocytes. Leukocytic counts on the blood were available in 18 of the 39 cases. Four cases showed 3,000 to 5,000 neutrophilic polymorphonuclear leukocytes per cubic millimeter. In 3 of these there was a moderate to marked shift to the left in granulopoiesis and the cellular pattern was normal in the other case. The remainder of the 18 cases showed a higher leukocytic count.

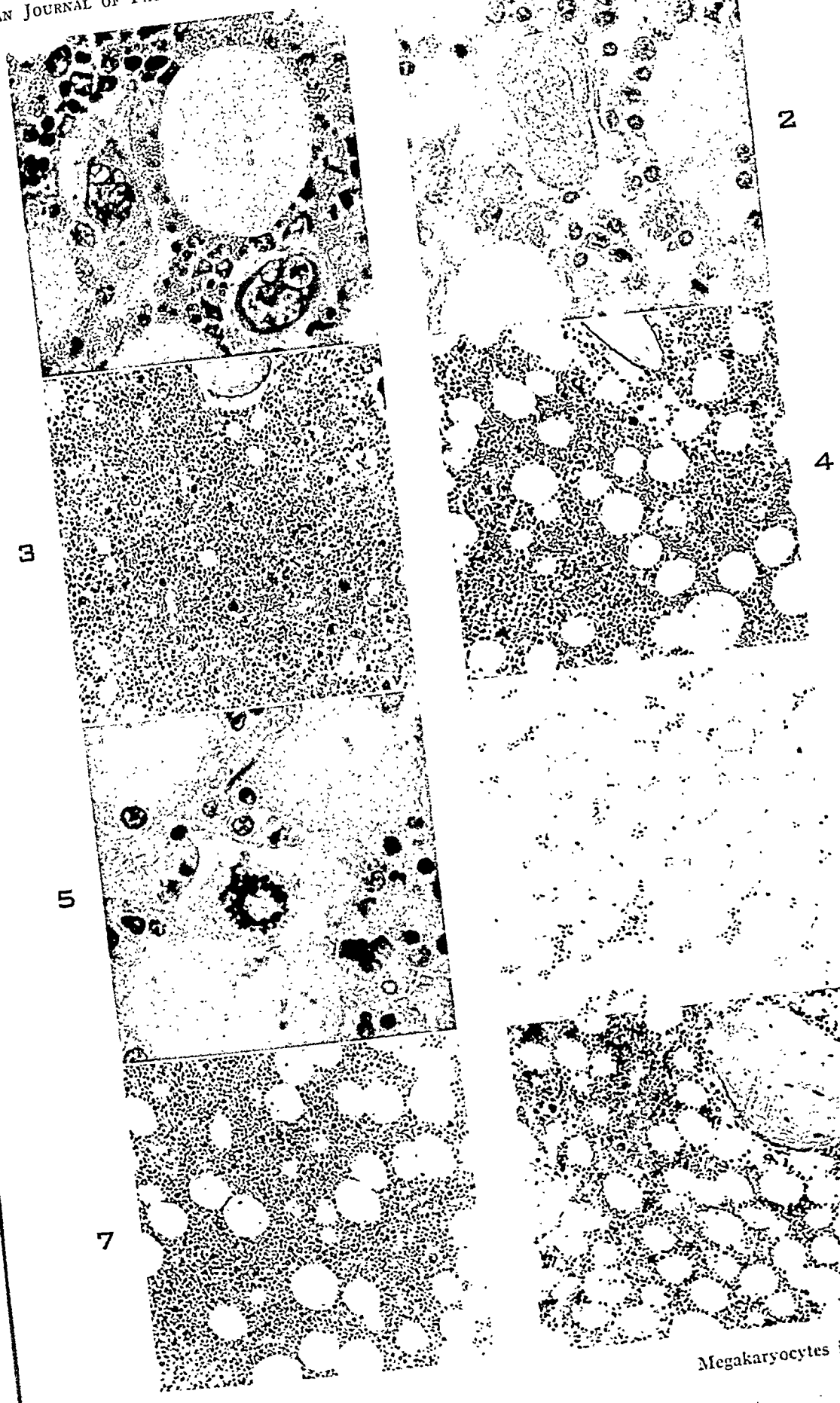
Summary

On the basis of inspection of the vertebral marrow and comparison with the controls, megakaryocytic hyperplasia was demonstrated in 26 of 306 unselected cases. Primary pneumonia occurred in 39, or 12 per cent, of the unselected cases whereas it occurred in 17, or 65 per cent, of the cases of megakaryocytic hyperplasia. This shows a definite relationship between pneumonia and hyperplasia of megakaryocytes. Counts of the megakaryocytes in the 39 cases of primary pneumonia revealed a number above the normal, *i.e.*, above 5,000 per cmm. of vertebral marrow, in 19 cases. There was no significant variation from the normal in the relative numbers of the types of megakaryocytes. Inspection of the marrow is a reliable method for the determination of an increase in the megakaryocytes in any series of cases.

In pneumonia there is a striking parallelism between hyperplasia of megakaryocytes and of leukoblastic cells. This megakaryocytic and leukoblastic hyperplasia occurred in infectious diseases in the absence of primary pneumonia and it is to be regarded as a type reaction. The hyperplasia begins first in the marrow of the lumbar vertebrae and only later takes place in that of the diaphysis of the humerus.

The study of the marrow of the vertebrae in contrast to that of the humerus or other long bones is necessary in order to detect and classify the reactions of the marrow.

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DESCRIPTION OF PLATES

PLATE 167

FIG. 1. Adult megakaryocytes of the rabbit. The cytoplasm is finely granular. There is a hyaline marginal zone. Wright's technic. × 570.

FIG. 2. Adult human megakaryocyte. There is a finely granular cytoplasm and a hyaline marginal zone. Modified Wright's technic. × 570.

FIG. 3. Pneumonia series. Case 31. Vertebra. A marked increase of megakaryocytes and of other hemopoietic cells is present. There is absence of definitive islands of erythropoiesis such as appear in Figures 8 and 9. Hematoxylin and eosin stain. × 100.

FIG. 4. Pneumonia series. Case 22. Vertebra. There is a moderate increase of megakaryocytes. The degree of hemopoietic cellularity is near the upper limit of normal. Well defined islands of erythropoiesis are absent in contrast with the marrow shown in Figures 8 and 9. Hematoxylin and eosin stain. × 100.

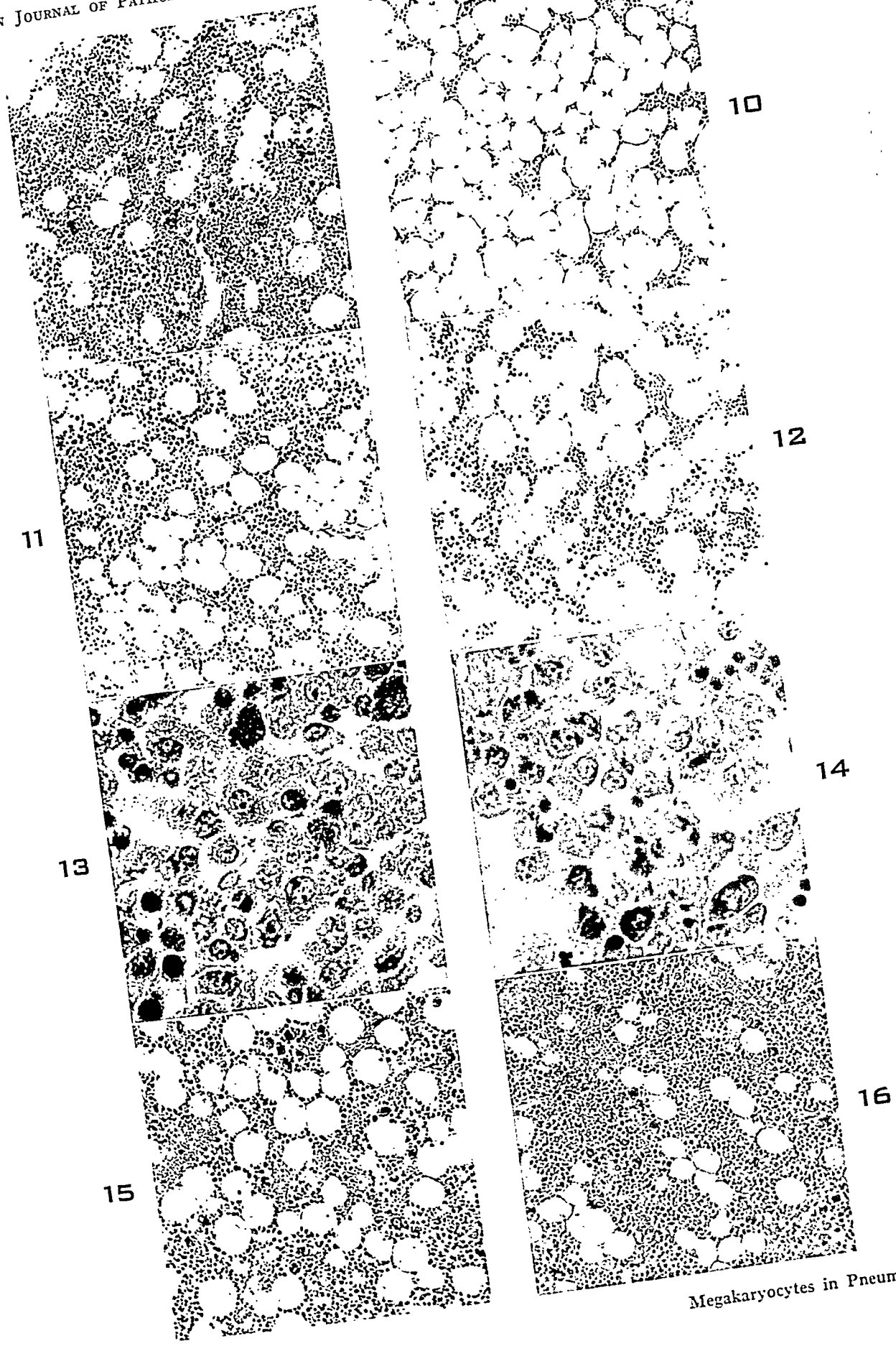
FIG. 5. Pneumonia series. Case 27. Humerus. A megakaryocyte with a bizarre mitotic figure. Hematoxylin and eosin stain. × 500.

FIG. 6. Control series. Case 12. Humerus. The marrow is completely fatty. The cells present are erythrocytes. Hematoxylin and eosin stain. × 100.

FIG. 7. Control series. Case 1. Humerus. This represents the maximum normal degree of hemopoietic cellularity for this location. Four megakaryocytes are present. Hematoxylin and eosin stain. × 100.

FIG. 8. Control series. Case 3. Vertebra. About one-fourth of the area is occupied by hemopoietic cells. Islands of erythropoiesis, composed of closely grouped cells with small black nuclei, are prominent. Hematoxylin and eosin stain. × 100.

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PLATE 168

FIG. 9. Control series. Case 10. Vertebra. About three-fourths of the area is occupied by hemopoietic cells. This is the upper limit of normal. The islands of erythropoiesis identified as closely packed small black nuclei are frequent and well defined. Hematoxylin and eosin stain. × 100.

FIG. 10. Control series. Case 12. Vertebra. A fatty focus in an otherwise active marrow. Hematoxylin and eosin stain. × 100.

FIG. 11. Control series. Case 15. Humerus. The islands of erythropoiesis are few in number and are ill-defined. Hematoxylin and eosin stain. × 100.

FIG. 12. Control series Case 16. Humerus. The islands of erythropoiesis, composed of closely packed cells with small black nuclei, are frequent and prominent as compared with Figure 11. Hematoxylin and eosin stain. × 100.

FIG. 13. Control series. Case 6. Vertebral pulp. The predominant cell of granulopoiesis is the mature neutrophilic myelocyte, which is identified by its cytoplasm being packed with fine granules. Azure II and eosin stain. × 777.

FIG. 14. Pneumonia series. Case 33. Vertebral pulp. The predominant cell of granulopoiesis is myelocyte A. Its cytoplasm either lacks granules or contains only a few as compared with the mature myelocytes shown in Figure 13. Azure II and eosin stain. × 570.

FIG. 15. Pneumonia series. Case 25. Humerus. The degree of cellularity in general is within the range of the controls. There is an increase in megakaryocytes as compared with the normal marrow of Figure 7. Hematoxylin and eosin stain. × 100.

FIG. 16. Pneumonia series. Case 31. Humerus. There is a marked increase in the total cellularity and in the number of megakaryocytes. Hematoxylin and eosin stain. × 100.

STUDIES ON FOWL LEUKOSIS *
TRANSFER TO THE CHICK EMBRYO

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The purpose of this paper is to report experiments with fowl leukosis, planned to determine: (1) whether fowl leukosis could be transmitted to the embryo by inoculation of the chorioallantoic membrane of the developing egg with blood, tissue and tissue filtrates of affected fowls, and (2) whether the agent of fowl leukosis can be cultivated and intensified by growth on the membrane of the developing egg.

Attempts at cultivation of the virus of fowl leukosis have been made by a few investigators. The tissue-culture technic for cultivation of virus was used by Furth and Stubbs (1934), Verne, Oberling and Guérin (1936) and Furth and Breedis (1937). In these experiments leukotic tissues were incubated in Carrel flasks, for varying periods of time, and the presence of the virus tested by inoculation of fowls with the cultures. Each of these observers concluded that, although the virus could be maintained for a short period while the growth of leukotic cells was maintained, cultivation of the agent had not been obtained.

Ruffilli (1937) was the first observer to claim that the virus could be cultivated in cultures of fibroblasts from heart muscle, without the presence of hematopoietic cells. Doljanski and Pikovski (1940) reported a small number of experiments which will bear confirmation since they are at variance with more extensive investigations. They reported successful takes in young fowls inoculated with tissue culture of embryonic heart muscles, bathed for 1 hour in a Berkefeld filtrate of leukotic spleen and subsequently grown in chick plasma in Carrel flasks. The cultures were incubated 2 and 4 weeks; of 16 chickens inoculated with these cultures, 15 were reported to have developed leukosis 12 and 20 days later. They also reported successful takes among fowls inoculated with washings of the original culture, maintained by transfer for 86 days; the washings produced leukosis in inoculated fowls even after the culture had undergone fibroblastic transformation.

At the time the studies reported in his paper were started, Jármai (1935) was the only investigator who had attempted to transmit leukosis to chicks by inoculating the egg with the virus. His attempts were successful only when eggs were inoculated after the ninth day of incu-

*This study was made possible by grants from the Elizabeth McCormick Fund of the Institute of Medicine of Chicago, the Edward Shedd Wells Memorial Fellowship Fund of The Children's Memorial Hospital, and the Otho S. A. Sprague Memorial Institute, Chicago.

Received for publication, February 2, 1942.

blasts, and (3) leukemic proliferation of the perivascular mesenchyme. Uninoculated embryos and embryos inoculated with normal chick leukocytes and spleen pulp served for controls.

Criteria of leukemic change in the older chickens were: (1) pale comb, (2) hemocytoblastosis and (3) characteristic leukemic infiltration in the organs.

I. Transmission to the Embryo with Spleen Pulp, Blood Leukocytes, Unfiltered Plasma and Berkefeld Filtrates of Spleen Extracts and Plasma

A. Transmission Experiments with Viable Cells from Spleen Pulp and Blood

Experiment 1. Spleens of leukotic fowls were removed aseptically, minced in Tyrode's solution, cultured for sterility and a small bit, 2 mm. in diameter, explanted onto the chorioallantoic membrane. In 45 per cent of the embryos examined after 20 days of incubation leukemic changes were found in the organs.

Table I

Percentage of Takes in Embryos Inoculated with Leukotic Spleen Pulp

Test number	Age of embryo when inoculated	Number of eggs inoculated	Number alive on 20th day of incubation	Embryos showing pathological changes of leukosis	Percentage of takes among embryos surviving experiment
1	10 days	12	3	1	33%
2	9 days	19	2	1	50%
3	10 days	16	3	3	100%
4	9 days	12	5	1	20%
5	9 days	12	1	1	100%
6	13 days	14	3	1	33%
Total		85	17	8	47%

Experiment 2. Leukocytes from the buffy coat of heparinized blood drawn from leukotic fowls were pipetted directly onto the chorioallantoic membrane. In two experiments all embryos which survived the incubation period showed characteristic leukemic changes in the liver, spleen and bone marrow.

Table II

Takes in Embryos Inoculated with Leukocytes of Leukotic Fowls

Test number	Age of embryo when inoculated	Number of eggs inoculated	Number of embryos alive on 20th day of incubation	Number of embryos fixed for histological study	Number of embryos allowed to hatch	Takes			
						Embryos		Hatched chickens	
						no.	%	no.	%
8	10 days	8	4	3	1	3	100	1*	100
11	9 days	11	2	2	0	2	100		
Total				5	1	5	100	1	100

* Died of leukosis at age of 5 weeks; blood leukocyte used for experiment.

bation. He concluded that, since bone marrow appeared at this time, the virus required bone marrow tissue for maintenance.

Since then, Kirschbaum, Stern and Hooker (1940) reported successful takes in 3 of 21 fowls 45 and 47 days old, which were inoculated with a Chamberland filtrate of leukotic plasma on the twelfth day of incubation of the developing egg. They presented no data regarding the actual cultivation of the virus. Using unfiltered plasma as the inoculum, they observed leukosis in chicks inoculated on the seventh day of the developing egg.

Storti and Mezzadra (1940) reported the transmission of leukosis to the embryo when the chorioallantoic membrane was inoculated with cell suspensions of leukotic tissue as early as the fourth day of development. They transmitted the disease to fowls by intramuscular inoculation of the embryonic tissue, of the first transmission, but were unable to prove the presence of the virus by similar inoculation of fowls after transmission to the second egg. They concluded that the virus could be maintained but not cultivated. However, since cell suspensions were used as the inoculum in their experiments, conclusions regarding the transmission of the virus must be guarded.

Method

A strain of leukotic fowl was developed for this experiment from leukotic fowls kindly supplied by Dr. Jacob Furth. Intravenous injections of leukotic blood or plasma into successive batches of young chickens maintained the strain. The developing egg technic, used by Rous and Murphy (1911) for study of fowl tumors, by Danchakoff (1920) for investigation of the effect of spleen grafts on the mesenchymal tissue of the embryo, and recently by Goodpasture and Anderson (1937), Burnet (1936) and numerous other investigators for the cultivation of viruses, was employed for the embryo experiments.

Fertile eggs were incubated at 39.5° C. for 9 to 11 days. A window 1.0 cm. square was then made in the shell with a dental drill, exposing the chorioallantoic membrane. The tissue or filtrate to be cultured was then placed on the membrane, the window rimmed with vaseline and sealed with a coverslip. The eggs were then reincubated for a variable number of days. Observations for gross change in appearance of the membrane were made through the coverslip; membranes and embryos were inspected for gross change before fixation in formaldehyde-Zenker's solution for histological study. Both membrane and embryos were sectioned and stained with hematoxylin and eosin.

The criteria of leukemic change in the embryo were: (1) intravascular and extravascular accumulations of hemocytoblasts; (2) replacement of the normal cellular content of the bone marrow by hemocyto-

Experiment 5. In three experiments, 16 embryos were allowed to hatch. One of the 16 developed leukosis after 5 weeks.

TABLE V

Leukosis Developing in Chickens Inoculated as Embryos with Leukotic Plasma

Test number	Inoculum	Age of embryo when inoculated	Number inoculated	Number hatched	Number of chickens developing leukemia	Inoculation period
9	Undiluted plasma	10 days	8	3	1*	5 weeks
16	Plasma diluted 3:1, filtered through Berkefeld V	10 days	12	6	0	
17	Plasma diluted 4:1, filtered through Berkefeld V	10 days	12	7	0	
Total			32	16	1	

* Plasma of this chicken transmitted leukosis to 4 of 5 chickens inoculated intravenously with 0.1 cc. of undiluted plasma, and 2 of 12 embryos inoculated on ninth day of incubation showed leukotic change in liver and bone marrow after 21 days of incubation.

C. *Transmission Experiments with Berkefeld Filtrates of Spleen and Plasma*

Experiment 6. Filtrates of tissue of leukotic spleen were prepared by passing a suspension of spleen pulps in Tyrode's solution through a gauze filter and then through a Berkefeld V filter. Cultures for sterility were made before and after filtration. The chorioallantoic membrane was inoculated with 0.5 to 1.0 cc. of the filtrate. In no instance was leukosis transmitted to the embryo by these filtrates.

TABLE VI

Results of Inoculation of the Chorioallantoic Membrane with Berkefeld Filtrates of Leukotic Spleens

Test number	Inoculum	Age of embryo when inoculated	Number of eggs inoculated	Number of embryos alive on 21st day	Embryos showing histological changes typical of leukemia
14	Splenic filtrate	9 days	24	6	0
15	Plasma filtrate	9 days	18	2	0
16	Plasma filtrate	9 days	12	8 (2 fixed)	0 (6 embryos hatched)
17	Plasma filtrate	10 days	12	7 (2 fixed)	0 (5 embryos hatched)
20	Splenic filtrate	10 days	10	2	0
21	Splenic filtrate	10 days	10	2	0
24	Splenic filtrate	11 days	12	3	0
Total				30	0

B. Transmission to the Embryo and to Older Chickens by Unfiltered Plasma

Experiment 3. Plasma was prepared by centrifugating leukotic blood at 6000 r.p.m. for 10 minutes. Two to 6 drops of the undiluted plasma were pipetted onto the chorioallantoic membrane. Five of 19 embryos which survived the 21-day period of incubation showed signs of leukosis.

TABLE III

Percentage of Takes in Eggs Inoculated with Plasma

Test number	Age of embryo	Number inoculated	Number of embryos alive on 20th day	Number of embryos fixed on 20th day	Number of embryos hatched	Takes			
						Embryos		Chickens hatched	
						no.	%	no.	%
7	10 days	8	6	3	3	0	0		0
9	10 days	10	4	2	2	0	0	1	50%
10	10 days	8	3	3	0	2	66%	0	0
11	11 days	12	3	2	0	2	100%	0	0
12	9 days	15	4	4	0	0	0	0	0
Total				14	5	4		1	

Total number of embryos alive at end of incubation period, 19.
Number and percentage of takes among embryos alive at end of incubation period, 4 (28%).
Number and percentage of takes after hatching, 1 (20%).

Experiment 4. Older chickens inoculated intravenously with undiluted plasma developed signs of leukosis more regularly than did the embryos, although in the individual experiments, when embryos survived the period of incubation, the percentage of takes was comparably high.

TABLE IV

Percentage of Takes in Older Chickens Inoculated with Plasma

Test number	Preparation of plasma	Dose	Age of chickens	Number inoculated	Number of takes	Takes	Incubation period
2	Centrifugated	0.5 cc.	6 weeks	4	3	74%	16, 40, 43 days
11*	Centrifugated	0.1 cc.	5 days	5	5	100%	29, 58, 76, 88 days
12	Centrifugated	0.1 cc.	2 weeks	4	1	25%	28 days
720	Centrifugated	0.2 cc.	3 weeks	4	2	50%	28 days
21	Centrifugated	1.0 cc.	6 weeks	4	1	25%	8 weeks
23	Centrifugated twice	0.5 cc.	6 weeks	3	1	33%	5 weeks
27a	Centrifugated	0.5 cc.	6 weeks	2	2	100%	26 days
5	Serum	0.5 cc.	6 weeks	4	0	0	
10	Serum	0.5 cc.	6 weeks	3	1	33%	5 weeks
Total				33	16	48%	

* Plasma produced typical leukosis in 4 of 5 five-day-old chickens.

infectivity of the filtrate. After incubation of the filtrate on the membrane for 4 to 6 days, the membranes were removed aseptically, pooled in Tyrode's solution, minced and ground, centrifugated for 5 minutes and 1 cc. of the supernatant fluid injected intravenously into another group of chicks. Evidence that the infectivity of the filtrates was increased by incubation on the membrane was not obtained.

Discussion

In the foregoing experiments, unfiltered suspensions and plasma of leukotic fowls produced leukosis in embryos and in chicks hatched from these embryos when placed on the chorioallantoic membrane after the eighth day of incubation of the egg. Berkefeld filtrates of leukotic tissue and plasma failed to produce the disease in eggs similarly inoculated. Failure of the filtrates to induce the disease in embryos or hatched chicks was not due to the noninfectivity of the filtrates, since they produced leukosis when injected intravenously into young fowls.

Failure of the filtrates to induce the disease in embryos might be due to (1) dilution of the agent in the process of filtration; (2) unsuitability of the chorioallantoic membrane for maintenance of the virus; (3) immunity of the embryo to certain concentrations of the virus. Since no evidence was obtained that the virus had been cultivated on the membrane, it is likely that the concentration of virus reaching the embryo was less than that provided by intravenous inoculation of the filtrate in young fowls. It is possible that the epithelium of the chorioallantoic membrane provides a barrier, and that intravenous inoculation of the embryo would be more successful. It is noteworthy that in the experiment of Kirschbaum, Stern and Hooker (1940) embryos developed leukosis when inoculated by injection of Chamberland filtrates of plasma into the albumin.

Failure of the virus to grow on the membrane may also be due to the unsuitability of the tissue for growth of the virus, and recalls the experiments of Furth and Stubbs (1934), Furth and Breedis (1937), Verne, Oberling and Guérin (1936), in which these investigators were unable to grow the virus in tissue culture without the presence of hematogenic cells. This controversial issue of the problem is heightened by the recent reports of Doljanski and Pikovski (1940) and Ruffilli (1937) who reported contradictory tissue culture experiments in which the virus did survive after the hematogenous cells had disappeared.

The histology of the membrane was not essentially changed by the presence of the filtrates or plasma. It remained transparent, unthickened and free of the focal opacities common to other virus infections

Experiment 7. Twenty-five per cent of the older chickens inoculated intravenously with these spleen filtrates developed leukosis.

TABLE VII

Percentage of Takes in Older Chickens Inoculated Intravenously with Berkefeld Filtrates of Leukotic Spleen and Plasma

Test number	Inoculum	Chickens		Takes		Incubation period
		Age when inoculated	Number inoculated	Number	%	
13	Splenic filtrate	3 weeks	4	2	50%	12 days, 10 weeks
15	Plasma filtrate	4 weeks	4	1	25%	10 days
17	Plasma filtrate	6 weeks	3	0		
20	Splenic filtrate	3 weeks	4	2	50%	6 weeks, 4 months
24	Splenic filtrate	6 weeks	3	0		
31	Splenic filtrate	6 weeks	4	1	25%	4 months
32	Splenic filtrate	6 weeks	2	0		
Total			24	6	25%	

II. TRANSMISSION TO CHICKS BY INOCULATION OF POOLED MEMBRANES PREVIOUSLY INOCULATED WITH FILTRATES OF LEUKOTIC TISSUE

In six experiments membranes were inoculated with Berkefeld filtrates of spleen pulp or plasma; in three instances young chicks were also inoculated intravenously with these same filtrates, to test the

TABLE VIII

Chickens Inoculated with Splenic Filtrates, and with Membranes Inoculated with These Same Filtrates

Experiment number	Inoculum	Takes among fowls inoculated directly with filtrate		Period of incubation after membranes were inoculated	Takes among fowls inoculated with saline suspension of membranes	
		No. inoculated	Takes		No. inoculated	Takes
22	Berkefeld filtrate of spleen–643	6	1	6 days	3	0
25	Berkefeld filtrate of plasma–644	none		10 days	3	1
26	Berkefeld filtrate of spleen no. 23	2	0	6 days	3	0
27	Berkefeld filtrate of plasma–648	2	1	8 days	2	0
33	Berkefeld filtrate of plasma–159	none		48 hours 5 days	3 3	0 0
34	Berkefeld filtrate of plasma–196	none		4 days	5	1
Total		10	2		22	2

follow than those in the liver and the data are insufficient to prove that the bone marrow was primarily involved. In the embryos inoculated with cell suspensions, the presence of foreign hemocytoblasts in the marrow confused the picture. In those inoculated with plasma, the marrow was predominantly hemocytoblastic by the time characteristic early changes were evident in the liver and before hemocytoblastosis developed.

Summary

Experiments are reported in which leukosis was produced in embryos, as well as in young fowls, by inoculation of the chorioallantoic membrane with unfiltered suspensions of leukotic spleen cells, leukocytes and plasma. Leukosis did not develop in embryos inoculated with Berkefeld filtrates of these tissues, although they were infective for young chicks. Suspensions of the membranes inoculated with the filtrates, and incubated for variable lengths of time, failed to produce leukosis in fowls by intravenous inoculation.

Conclusion

While evidence was not obtained that the virus of chick leukosis can be cultivated on the chorioallantoic membrane of the developing egg, the disease can be transmitted to the chick embryo by inoculation of the egg with tissues of leukotic fowls.

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of the membrane. Necrosis of the epithelium did not occur, inclusion bodies were absent and the cellular reaction was no more pronounced than it was in membranes inoculated with sterile broth or saline (Fig. 1). This is quite different from the histological picture of membranes inoculated with cell suspensions of leukotic tissue, which produce gross thickening and cloudiness of the membrane. The thickening is due to intercellular edema, and the opacity to the marked cellular reaction.

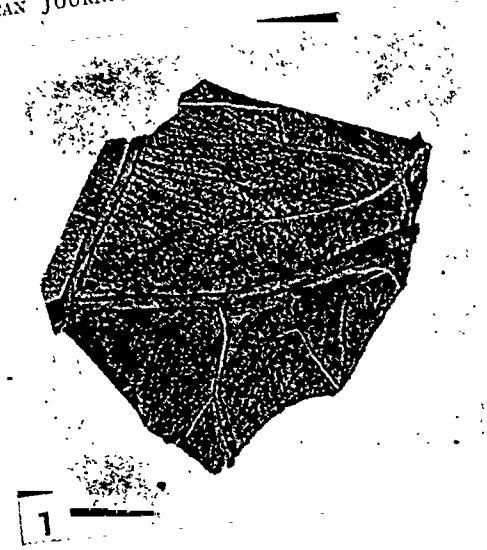
The explanted cells of leukotic spleen readily formed grafts which in some instances hypertrophied to several times the size of the original explant (Figs. 2 and 3). Shortly after explantation, the actively ameboid leukemic cells wandered into the membrane away from the explant and mingled with the normal cellular constituents, *i.e.*, neutrophils, lymphocytes and polyblasts. The leukemic cells in various stages of mitosis and disintegration could be traced into the blood capillaries of the membrane and thence into the embryo to the sinusoids of liver and bone marrow. After 48 to 72 hours, the explant was walled off by polyblasts, canalized by blood vessels and a true graft was established. The process was essentially the same as with normal spleen grafts, described by Danchakoff (1920), except that the hypertrophy of the graft was more extensive and rapid, due to the active proliferation of the leukemic cells.

The explanted leukemic blood leukocytes behaved in a manner similar to those of the spleen pulp, except that they did not form grafts, but were rapidly dispersed throughout the membrane and thence into the sinusoids of the embryo. The chorioallantoic membrane provides a culture medium for the maintenance of the leukemic cells, a certain number of which survive, migrate by ameboid motility throughout the membrane and, via the blood vessels, to the embryo where they set up metastatic foci of cell formation.

That the pathological findings of leukosis in the embryo are entirely due to these transported cells seems likely, but, until the nature of the agent is known, this remains a question. The earliest leukemic change was most easily recognizable in the liver, where clumps of hemocytoblasts were first seen in the smaller sinusoids and among the perivascular cells. In the older embryos, the lesions were more typical and were characterized by large aggregates of stem cells throughout the sinusoids of the liver, by intravascular hemocytoblastosis and by wide zones of stem cells (Fig. 5). The embryos showing the most extensive leukemic change were those inoculated with cell suspensions, but those inoculated with plasma also showed convincing, although less striking, changes in the bone marrow and perivascular mesenchyme (Fig. 6).

Early leukemic changes in the bone marrow were more difficult to

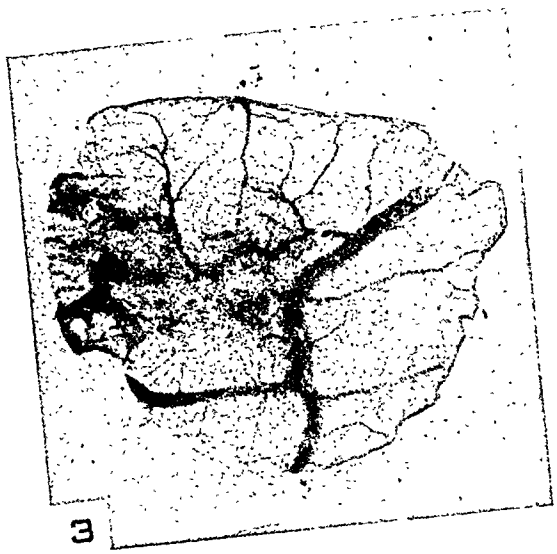
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DESCRIPTION OF PLATES

PLATE 169

FIG. 1. Photograph of chorioallantoic membrane inoculated on the seventh day of incubation with 10 drops of sterile broth, showing small focal opacities due to collections of inflammatory cells in the mesenchyme.

FIGS. 2 and 3. Photographs of chorioallantoic membranes 9 days after inoculation with minced spleen pulp of leukotic fowl, showing gross hypertrophy of the spleen explant.

FIG. 4. Chorioallantoic membrane inoculated on the 9th day of incubation with leukocytes of a leukotic fowl (no. 3) and fixed for histological examination on the 20th day of incubation. Above and to the right there is a clot of disintegrating explanted leukocytes. The ectoderm is thickened, and there is a cellular reaction within the mesenchyme. × 45.

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Studies on Fowl Leukosis

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PLATE 170

FIG. 5. Liver of an embryo, the chorioallantoic membrane of which was inoculated with leukocytes of a leukotic fowl (no. 3) (see Fig. 4), showing aggregation of stem cells about a central vein and also within the lumen of the vein. × 715.

FIG. 6. Liver of an embryo, the chorioallantoic membrane of which was inoculated with plasma of a leukotic fowl (no. 3) (see Fig. 7), showing a cluster of stem cells within an hepatic sinusoid and vacuolization of liver cells. × 715.

FIG. 7. Chorioallantoic membrane inoculated on the 9th day of incubation with 10 drops of plasma of a leukotic fowl (no. 3), showing edema and cellular reaction in the mesenchymal layer of the membrane. × 70.

EFFECTS OF THE "GROWTH HORMONE" OF THE ANTERIOR HYPOPHYSIS (ANTUITRIN G) ON THE SKELETON OF MICE AND GUINEA PIGS*

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In previous experiments in growing mice and guinea pigs [1-4] we found that acid extracts of the bovine anterior hypophysis, similar to those used by Loeb and Basset [5] in their experiments, induced a temporary stimulation of the proliferation of the epiphyseal cartilage followed by an acceleration and a premature onset of ageing processes in the skeletal tissues. Syngenesiotransplants of mouse anterior hypophysis,[4] on the other hand, caused a temporary inhibition of the growth of the cartilage associated with a prolongation of the growth period. This likewise was followed later by an acceleration of skeletal ageing. In neither case was gigantism observed, nor did the degree of the age changes in the epiphyseal disks surpass the maximum of such changes occurring in normal old animals.

According to Ray, Evans and Becks,[6] Thomson and Collip [7] and some other investigators,[8,9] the anterior hypophyseal gland contains a growth-promoting substance that can be extracted by alkaline, but not by acid solutions. It might, therefore, be assumed that the acid extracts of anterior hypophysis used in our previous investigations did not contain this growth substance. For this reason, we decided to compare the action of an alkaline extract (Antuitrin G) on bone and cartilage of mice and guinea pigs with the action of acid extract employed in our earlier investigations. Antuitrin G is made specifically to obtain a potent preparation of the growth-promoting principle of the anterior hypophysis.

MATERIAL AND METHODS

Forty-three mice and 8 guinea pigs were used in these experiments. One group of mice consisted of growing animals 5 to 6 weeks old at the beginning of the experiments; the other of adult animals, 3 to 4 months old, in which the growth of the cartilage had become very slow or was about to cease altogether. In the first group, 10 mice of strain C_{57} (9 males and 1 female) and 6 of strain D (5 females and 1 male)

*These investigations were carried out with the aid of grants from the International Cancer Research Foundation, from the Jane Coffin Childs Memorial Fund for Medical Research and from the Committee on Research in Endocrinology of the National Research Council.

Received for publication, February 6, 1942.

advancing from the bone marrow and their rapid replacement by osteocytes. In some area, ossification had proceeded so rapidly that unopened hypertrophic cartilage cells became incorporated in the newly formed trabeculae. Regressive changes had set in and amorphous plugs of degenerated cartilage were visible traversing the epiphyseal plate

TABLE I

Weights of the Control Animals and of the Animals Injected with Antuitrin G, in Grams

Growing mice

Duration of experiment	Mice of strain C57				Mice of strain D			
	Controls		Injected		Controls		Injected	
	Init.	Final	Init.	Final	Init.	Final	Init.	Final
1 week	14.5	19.0	14.5	16.3	17.2	19.4	16.0	16.2
			13.5	15.0			16.0	18.7
2 weeks	13.5	15.2	13.0	16.0	12.0	12.6	12.5	16.6
			13.5	14.8			10.0	14.9
4 weeks	13.5	22.5	15.0	21.9	9.0	13.5	9.0	16.2
			15.0	20.8			12.0	18.4
2 months	15.5	22.0	15.0	24.5				
			13.0	24.5				
3 months	16.5	28.0	14.0	27.9				
			12.5	23.2				

Adult mice

Duration of experiment	Mice of strain C57				Mice of strain D			
	Controls		Injected		Controls		Injected	
	Init.	Final	Init.	Final	Init.	Final	Init.	Final
1 week	20.0	22.0	20.5	20.5	19.2	18.5	17.5	20.0
			22.0	28.1			19.3	20.9
2 weeks	20.5	20.5	21.5	24.4	18.8	18.8	19.0	20.0
			21.5	25.3			16.7	19.9
4 weeks	22.0	26.7	22.0	27.7	18.0	18.7	17.5	21.7
	20.5	22.7	23.0	27.7			17.5	22.8

Guinea pigs

Duration of experiment	Controls		Injected	
	Init.	Final	Init.	Final
4 days	175	185	205	172
	175	190	180	177
14 days	198	253	185	193
	175	215	177	176

(Figs. 1 and 2), an occurrence usually not found in healthy mice of the C_{57} strain before the end of the fourth month of life.

In the subepiphyseal layer, the peritrabecular connective tissue cells were converted into epithelioid cells in greater numbers than ordinarily. These latter cells arranged themselves in a beadlike manner along the surface of the spicules and acted as osteoblasts; as a consequence, numerous thick, bony trabeculae were laid down.

received subcutaneous injections of 1 rat unit (0.1 cc.) of Antuitrin G three times weekly for periods of 1, 2 and 4 weeks, and 2 and 3 months. In the older group, 6 male mice of strain C_{57} and 6 females of strain D received 1.5 rat units (0.15 cc.) of Antuitrin G three times weekly for periods of 1, 2 and 4 weeks. Nine male mice of the C_{57} strain and 6 females of the D strain of corresponding age served as normal controls. Littermates were used in all groups as far as possible.

Furthermore, 2 female guinea pigs weighing 180 to 200 gm. received daily intraperitoneal injections of 10 rat units (1 cc.) of Antuitrin G for 4 days and 2 others were treated in the same way for 14 days. Four guinea pigs of similar age and weight served as controls.

After necropsy, the endocrine organs, tibia and femur, and, in the case of guinea pigs, the chondro-osseous junctions of several ribs were prepared for histologic study, as described for previous experiments.[2,10]

Observations

In Table I the initial and final weights of each animal are given. Since most of the animals within a group were littermates, the differences within the individual groups may be more indicative of the changes in weight under the given experimental conditions than means based upon a greater number of unrelated individuals.

In the growing mice, the increase of weight during the first and second weeks of the experiments did not surpass that of the controls; there was, on the contrary, rather a slight tendency towards a lesser gain of weight in the treated group. From about the fourth week of treatment this tendency was reversed, and the injected mice gained weight more rapidly than their controls. In the older group, the injected animals showed a greater increase of weight than their controls during the period of observation. Whereas the control guinea pigs gained weight steadily, the injected guinea pigs showed at first a loss, the initial weights being regained after about 2 weeks of treatment.

Histologic Examination

Growing Mice of Strain C_{57} 5 to 6 Weeks Old at the Beginning of the Experiment

After 1 week of injections, the growth zone of the upper tibia was somewhat narrowed, but contained many cartilage cells which were more closely packed in the single rows than usual. The ground substance was diminished. The dispersed nonoriented cartilage cells were quickly converted into columnar cartilage cells which exhibited frequent mitotic division. The layer of hypertrophic cartilage cells was narrower than normal due to an intensified breakdown by capillaries

However, even after 3 months of the treatment there were only small perforations of the epiphyseal zone, and no extensive epiphyseo-diaphyseal union had occurred. No alterations of the articular cartilage were noted. In the bony shaft, increased vascular and cellular resorption had caused a removal of the excess bone found at the earlier stages of the experiment. In some instances this resorption was so marked that the compacta appeared even thinner than in untreated animals of corresponding age.

Mice of Strain C_{57} 3 Months Old at the Beginning of the Experiment, and Approaching the End of the Growth Period

One week after the beginning of the injections of Antuitrin G the width of the epiphyseal growth zone did not differ greatly from that in corresponding noninjected mice. The number of cartilage cells in a single cartilage cell row was not increased and there was no evidence of a stimulation of the proliferation of the cartilage cells as seen in younger animals at the corresponding stage of the experiment. However, many of the more proximally situated cartilage cells had assumed a vesicular appearance, indicating that these cells had undergone an increased hypertrophy. The layer of the hypertrophic cartilage cells proper was narrower than usual due to a rapid ossification. Because of increase in the size of the cells, the cartilaginous ground substance appeared diminished. Hand in hand with the increased hypertrophy of the cartilage cells went regressive changes, leading to the destruction of cartilage cell rows and to the appearance of fairly thick, amorphous plugs of degenerated cartilage in the epiphyseal plate. After 2 weeks of injections, there was likewise no noticeable stimulation of cell multiplication in the epiphyseal cartilage, but regressive changes of the latter were intensified (Figs. 3 and 4). The cartilaginous matrix and the cartilage cells were increasingly calcified and the ossification of the hypertrophic cartilage was accelerated. The amorphous plugs in the epiphyseal disk were thicker than after 1 week of treatment, and had undergone further calcification and replacement by bone. Vascular and cellular resorption became more and more pronounced, causing a dissolution of the osseous plugs and of the osseous spicules to such a degree that in some places only a thin bony plate separated the epiphyseal cartilage from the diaphyseal marrow. Similar changes produced a thinning of the bony border lamella separating the articular cartilage from the epiphyseal marrow. The cartilage cells of the joint showed hyperplastic and hypertrophic changes and underwent regression. The cartilaginous ground substance was softened and fibrillar; but no severe arthropathic lesions were produced (Figs. 5 and 6). The peri-

The articular cartilage cells underwent an accentuated proliferation associated with increased mitotic division, as well as with intensified hypertrophy and accelerated ossification.

The periosteal and endosteal cells were increased in number, and, in contact with the compacta of the shaft, produced more bone than normal. The vascular canals were narrow, and cellular resorption was less marked than in untreated mice.

After 2 weeks of administration of Antuitrin G, the epiphyseal disk was greatly narrowed and the proliferation of the epiphyseal cartilage had decreased. Calcification and ossification had increased further. Simultaneously, however, processes of resorption acting on cartilage and bone were intensified. There was further narrowing of the epiphyseal growth zone and a thinning and solution of the subepiphyseal trabeculae. In the joint, some cartilage cells had undergone liquefaction.

After 4 weeks of injections of Antuitrin G, the epiphyseal growth zones were distinctly narrower than normal. The cartilaginous matrix and the cartilage cells were heavily calcified. The cells were atrophic, their size being reduced about one-half, but the number of cartilage cells in a single cartilage cell row did not deviate greatly from that of the controls. Proliferation of these cartilage cells was not observed. However, the processes of ossification still appeared accelerated and intensified. The subepiphyseal trabeculae continued to be fairly thin and short and showed transverse links. Thus, in mice 2½ months old, that had been injected for 1 month, a transverse bony plate began to form separating the calcified epiphyseal cartilage from the bone marrow, a condition usually not found in untreated mice of strain C_{57} before the age of 4 to 5 months.

In the articular cartilage, the proliferative and regressive changes receded gradually. In the shaft, the osteocytes were larger than at earlier stages of the experiment and the osseous substance was softer. The vascular canals were enlarged and the cellular resorption of bone by the periosteum was also increased.

After 2 months of injections the epiphyseal disks were narrow and strongly calcified. The cartilage cell columns were short. The persisting cartilage cells, the cartilaginous ground substance and the degenerative plugs, that in the meantime had increased in number and extent, were densely calcified. There was no evidence of proliferation of the cartilage cells. The transverse osseous plate underneath the hypertrophic cartilage was thickened as compared with that at the end of 1 month of injection, but resorption of the bony material as well as of the calcified and partly ossified plugs had also made progress.

Antuitrin G for 4 weeks corresponded to that seen in normal mice of this strain at the age of 10 to 12 months. In mice of strain C_{57}, the structural age of the epiphyseal cartilage was thus advanced by about 6 to 8 months, which is about three times as much as in mice of strain D.

Even in littermates, treated or untreated, individual differences as to the progress of skeletal ageing and of the weight curves exist.

Guinea Pigs

After 4 days of injections of Antuitrin G, the epiphyseal growth zone of the upper tibia in guinea pigs was somewhat narrower than usual. The number of cartilage cells in a single cell column was decreased as compared with the normal. The cartilaginous ground substance was swollen and vacuolated. The columnar cartilage cells had undergone a marked mitotic proliferation and rapid hypertrophy, and, as was the case in young mice, the zone where the hypertrophy of the cartilage cells began was shifted farther proximally. The largest cartilage cells of the hypertrophic type present were smaller than usual, the largest ones being replaced by bone as soon as they had been formed. In the subepiphyseal layer, instead of a richly vascularized hemopoietic marrow, a cellular connective tissue had developed in which numerous mitoses indicated the degree of stimulation exerted on the connective tissue cells. A great number of these cells had been converted into mitotically proliferating osteoblasts, whereas others participated in resorptive processes which were intensified. In these two antagonistic processes the former was predominant for there was an increase in the number of trabeculae. The articular cartilage cells were likewise hyperplastic and hypertrophic. There was a marked increase of cells in the periosteum.

Two weeks subsequent to the beginning of the administration of Antuitrin G, the proliferation of the epiphyseal cartilage was still somewhat intensified. However, in the meantime, regressive and resorptive processes had also made progress and had begun to predominate over the growth processes. Ossification had likewise markedly progressed. Because of the increase in formation as well as in resorption of bone, the subepiphyseal tissue showed an irregular appearance. In some places, fibrous tissue had replaced the excessive number of trabeculae found previously and had penetrated through the cartilaginous disk into the epiphyseal marrow cavity. In other areas, strands and islets of unopened columnar cartilage cell capsules were lying far distally in the metaphysis, enclosed by osseous tissue.

In the articular cartilage, hyperplastic and hypertrophic processes were associated with increased resorption leading to a thinning and

osteal tissue was loose, and increased vascular and osteoclastic resorption led to a thinning of the bony shaft.

After 4 weeks of treatment, the conditions were similar to those seen after 2 weeks, but, on the whole, the resorptive processes were more marked than the degenerative changes of the cartilage.

Mice of Strain D, 5 to 6 Weeks Old at the Beginning of the Experiment

As reported previously,[10] the skeletal tissues of mice of strain D exhibit normally a faster rate of skeletal ageing than those of strain C_{57}. Thus in untreated mice 8 weeks old of strain D, the growth zones appeared only slightly younger than those of a mouse 4 months old of strain C_{57}.

After 1 week of administration of Antuitrin G, there was somewhat increased proliferation of the epiphyseal cartilage, which, however was less accentuated than in corresponding mice of strain C_{57}. As compared with the latter, the tendency of the cartilage cells of mice of strain D to undergo hypertrophy, retrogression and ossification was likewise less intense, but these processes were still increased over the normal.

After 2 weeks of injections, proliferation of the cartilage cells had increased slightly or not at all, whereas hypertrophy and degeneration of these cells had progressed further. After 4 weeks of injections, the retrogressive changes in the cartilage and the formation and resorption of bone were slightly advanced over the normal; in isolated areas, perforations of the epiphyseal disk were initiated.

Mice of Strain D 3 Months Old at the Beginning of the Experiment

One and 2 weeks subsequent to the beginning of the administration of Antuitrin G there was no evidence of a stimulation of the proliferation of the epiphyseal cartilage, that normally at this age is already in an inactive state. But the regressive changes taking place in the cartilage were somewhat advanced over the normal. Resorptive processes acting on cartilage and bone were likewise intensified. After 4 weeks of treatment in mice of strain D, that were then 4 months old, advanced resorptive processes had led to fairly wide perforations of the epiphyseal disk. This may seem at first to be a more marked effect than that exerted by Antuitrin G in corresponding mice of strain C_{57}, in which only small perforations of the epiphyseal zone were present, but the condition produced in mice of the D strain was comparable to that seen in normal mice of this strain at the age of 6 to 7 months, and thus the structural age of the cartilage was increased by about 2 to 3 months in these mice. In mice of strain C_{57}, on the other hand, the structure of the epiphyseal zone in animals 4 months old treated with

terior hypophyseal extract. Whereas perforations of the epiphyseal disk could be initiated after 2 weeks of injections of Antuitrin G, even under prolonged treatment with anterior hypophyseal extract increased amounts of bone could still be present in the diaphysis and in the beaded chondro-osseous junction of the ribs.[3,11] This delay of the progress of resorptive processes may, however, be correlated with the decrease of effectiveness of the acid hypophyseal extract in the course of prolonged administration.[3] Under certain conditions that are now being investigated, resorptive processes are likewise resumed in guinea pigs under the influence of acid anterior hypophyseal extract, leading to a removal of the excessive amounts of bone.

Antuitrin G as well as acid anterior hypophyseal extract may accelerate every phase of skeletal growth and ageing, the phases affected in each single instance being those that are in progress at the beginning of the treatment. In other words, Antuitrin G, like other hormones, alters the time curve of the tissues on which it acts (Loeb [12]). No evidence could be obtained from the present investigations that in normal animals Antuitrin G stimulates the growth of the cartilage beyond its normal limits. In growing animals the temporary increase of proliferation is balanced by a shortening of the period during which growth takes place, and results in an accelerated exhaustion of the growth capacity of the cartilage. In animals in which the growth capacity of the cartilage has been naturally exhausted, no resumption of proliferation of cartilage could be effected by the administration of Antuitrin G. The temporary character of the growth stimulation of the epiphyseal cartilage as well as the lack of growth stimulation in adult animals explain the failure of this substance to produce gigantism.

Growing mice receiving Antuitrin G did not weigh more than did the control mice and in many instances weighed less. The guinea pigs treated with Antuitrin G showed an initial weight loss. A greater gain in weight of the injected mice as compared with their controls became noticeable only after the growth period was terminated. This occurred in animals in which the growth period had ended prematurely due to the injections of Antuitrin G as well as in animals which had reached the end of the growth period, when the treatment was begun. In the latter case, the increase of weight began immediately after the first injection and might have been largely due to storage of fat (Evans and Long [13]) or to an accumulation of water by the tissues (Downs [14]).

The difference in the response of the weights of growing and adult mice might be connected with the different reactions of the thyroid gland to Antuitrin G at different ages. Loeb [15] studied the thyroid glands of the mice and guinea pigs used in these investigations and

corrosion of the bony border lamella separating the epiphyseal cavity from the articular cartilage. The cells at the inner and outer surface of the compacta of the shaft proliferated. The vascular canals were enlarged, and the resorption of bone began to become conspicuous. At the chondro-osseous junction of the ribs the changes observed were similar to those at the growth zones of tibia and femur.

Comment

Antuitrin G administered subcutaneously to growing mice and guinea pigs increased temporarily the proliferation of the growing cartilage, accelerated its hypertrophy and caused a premature onset and an acceleration of degenerative and resorptive processes in the epiphyseal growth zones. Formation of bone in the subepiphyseal tissues and in the shaft was temporarily increased, but this increase was followed by enhanced processes of resorption. Antuitrin G also called forth hyperplastic, hypertrophic and slight regressive changes in the articular cartilage but it did not produce severe arthropathic lesions.

In adult mice, 3 to 4 months old, in which the growth capacity of the epiphyseal cartilage is almost exhausted, Antuitrin G failed to induce any proliferation in the epiphyseal cartilage. However, the columnar cartilage cells, which were for the greater part preserved at the beginning of the treatment, underwent an accelerated degeneration and calcification followed by breakdown and ossification. In the subepiphyseal tissues and in the shaft, resorption of bone became prominent. In the joint, hyperplastic and hypertrophic changes of the cartilage were associated with regressive processes, but likewise without the occurrence of severe lesions.

Antuitrin G is more effective in accelerating the skeletal age changes in mice of strain C_{57}, which normally show a slow rate of skeletal ageing, than in mice of strain D, the skeleton of which ages more rapidly under normal conditions.[10] On the whole, therefore, Antuitrin G exerts on the skeleton effects similar to those exerted by acid extracts of the bovine anterior hypophysis formerly used by us, although, in the dose given, Antuitrin G had a weaker effect on the skeleton of mice than corresponding amounts of the bovine anterior pituitary extracts.

In immature guinea pigs, both Antuitrin G and acid anterior hypophyseal extracts are more effective on the skeletal tissues than in mice. Both substances stimulate temporarily the proliferation of the epiphyseal and articular cartilage and cause a premature onset of degeneration and an acceleration of ossification. Apparently, however, Antuitrin G intensifies resorptive processes more than does acid an-

to show that the latter hormone causes a delay in the ageing of the skeleton which is the opposite of the effect called forth by the acid hypophyseal extract.

Ross and McLean [9] found that Antuitrin G, injected intraperitoneally into rats 6 months of age, had no effect on the growth zones. The lack of proliferation of the epiphyseal cartilage in their rats corresponds to our findings in mice, which were 3 months old at the beginning of the treatment. In these mice we found no effect of Antuitrin G on the proliferation of the cartilage, but there occurred increased ageing of the skeletal tissues. It is, of course, possible that the ineffectiveness of Antuitrin G in rats in the experiments of Ross and McLean might have been due to species differences.

According to our findings, Antuitrin G may be included in the group of those hormones or hormonelike substances which, under certain conditions, can accelerate all three phases of skeletal growth and ageing; in this group belong also the anterior hypophyseal extracts,[4] thyroxin [23] and potassium iodide.[24] All of these substances [25] influence in a similar way the growth and ageing of the epiphyseal cartilage of normal animals. It seems doubtful, therefore, whether one is justified in stipulating that there is a single specific growth hormone produced by the anterior hypophysis, inasmuch as processes other than those of growth are affected by the same substances.

Summary

In growing mice and guinea pigs, Antuitrin G influences all three phases of skeletal growth and ageing. It causes a temporary stimulation of the proliferation of the epiphyseal cartilage, associated with a shortening of the growth period and followed by premature onset and an accelerated course of age changes such as degeneration, calcification and breakdown of the cartilage. Antuitrin G also promotes processes of bone formation as well as those of resorption of bone.

In adult mice which have reached the end of their physiologic growth period, Antuitrin G fails to call forth renewed proliferation of the epiphyseal cartilage. But such processes as are in progress at the time of the beginning of the experiment are accelerated under the influence of Antuitrin G.

The effects of Antuitrin G on cartilage and bone are, therefore, in principle, the same as those exerted on these tissues by acid anterior hypophyseal extract; the differences that do exist largely concern the degree of the changes produced, the anterior bovine hypophyseal extract being more effective in mice. Antuitrin G may thus be included in one group with various other anterior hypophyseal extracts, thy-

found in the growing animals, particularly in the guinea pigs, a marked thyrotropic effect of Antuitrin G, similar to that seen after administration of acid anterior hypophyseal extract of cattle; in guinea pigs this effect decreased with increasing duration of the treatment. Loeb and his associates [5,16,17] had furthermore shown that there was no essential difference in the effects of acid and alkaline extracts of the anterior hypophysis of cattle and sheep on the thyroid and ovary of the guinea pig.

There is a fundamental similarity in the histologic changes produced in the skeleton by the acid anterior hypophyseal extract and Antuitrin G. This similarity is of interest because it had been suggested by some investigators [7,8] that the acid anterior hypophyseal extract used by us in previous experiments was too crude for the demonstration of the action of the so-called growth hormone. Furthermore, the result obtained with the extract should not be comparable to those produced by the alkaline extract of anterior hypophysis.[9,18] However, recently, Ray, Evans and Becks,[6] after injecting purified alkaline hypophyseal extracts in rats for 10 days, reported a marked stimulation of endochondral ossification, atrophy of trabeculae and a less marked periosteal ossification. These histologic findings agree essentially with those we have previously observed at certain stages following injections of acid extracts of bovine anterior hypophysis; these changes are interpreted as part of the general acceleration of growth and ageing processes of the skeletal tissues. As to possible effects on the skeletal tissues of the gonadotropic fraction contained in the acid extract and which Ray, Evans and Becks believed to be active in producing our results, we may refer to one of our previous investigations.[2] In that study we investigated the influence of implants of the bovine anterior hypophysis on the epiphyseal cartilage of guinea pigs. One group of animals received the fresh glands; others received glands that prior to implantation had been treated in such a way as to suppress one or the other gonadotropic activity (Loeb and Hayward [19]). In experiments conducted as long as 6 days we were unable to establish any correlation between the reaction of the epiphyseal cartilage to the follicle-stimulating or luteinizing, or atresia-producing action of such implants.

The lack of any significance for these gonadotropic fractions in inducing an indirect effect on the epiphyseal cartilage is also indicated by the fact that anterior hypophyseal extract exerts its influence also in castrated animals.[20,21] Furthermore, the predominant gonadotropic fraction contained in the acid extract being the luteinizing hormone, a secondary effect which might be exerted by the intermediation of the ovaries should be similar to that of progesterone. We [22] were able

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roxin and potassium iodide, all of which influence in a similar way the growth and ageing processes in the skeletal tissues.

NOTE: We are indebted to Dr. Oliver Kamm of Parke, Davis & Co. for the Antuitrin G used in these experiments, and to Edgar Nebel for the photomicrographs.

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Skeletal Effects of Antuitrin G

Silberberg and Silberberg

DESCRIPTION OF PLATES

PLATE 171

FIG. 1. Section through the epiphyseal disk of the upper tibia of a normal male mouse of strain C_{57}, 7 weeks old, whose weight had increased from 14.5 to 19.0 gm. during 1 week of observation. × 125.

FIG. 2. Section through the epiphyseal disk of the upper tibia of a male littermate of the animal shown in Figure 1, which had been injected with 1 rat unit (0.1 cc.) of Antuitrin G three times weekly for 1 week. The weight had increased from 14.5 to 16.3 gm. Ossification of the epiphyseal cartilage has progressed farther than in Figure 1. There is a plug of degenerated cartilage in the epiphyseal plate. × 125.

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PLATE 172

FIG. 3. Section through the epiphyseal disk of the upper tibia of a normal male mouse of strain C_{57}, 16 weeks old; initial and final weights were 20.5 gm. for 2 weeks of observation. × 80.

FIG. 4. Section through the epiphyseal disk of the upper tibia of a male littermate of the animal shown in Figure 3, which had been injected with 1.5 rat units (0.15 cc.) of Antuitrin G three times weekly for 2 weeks. The weight had increased from 21.5 to 24.4 gm. during this period. Resorption of cartilage and of subepiphyseal trabeculae has advanced farther than in Figure 3. There is an ossified plug at the right margin of the epiphyseal plate. × 80.

FIG. 5. Section through the articular surface of the lower femur of the same animal as shown in Figure 3. × 230.

FIG. 6. Section through the articular surface of the lower femur of the same animal shown in Figure 4. Increased hyperplasia and hypertrophy of the articular cartilage and retrogressive changes of cartilaginous matrix and of some cartilage cells. × 230.

FIBROSIS OF THE PANCREAS IN MEAT-FED DUCKS*

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Pancreatectomy of domesticated ducks,[1] unlike that of owls,[2] does not result in the development of permanent hyperglycemia. The essentially herbivorous nature of the duck (in contrast to the carnivorous habits of the owl) made a study of the relation of the diet to the response to pancreatectomy of interest.

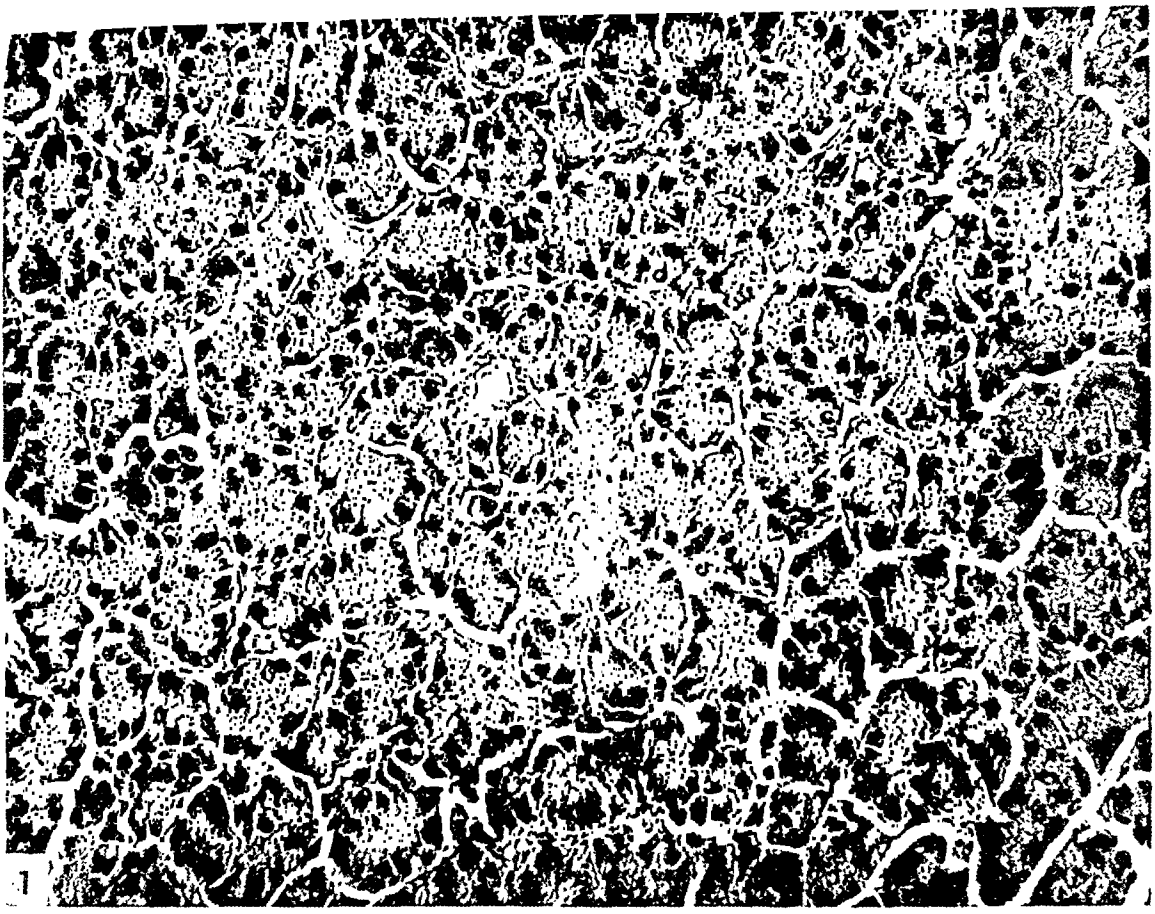
Beginning at 1 day of age, ducks were fed freely on a diet of raw ground meat (50 cc. of cod liver oil was added to each 8 lbs. of meat). Birds that attained an age of 6 months to 1½ years were used for this study. Each of these was depancreatized and subsequently its diabetic status was evaluated. A total of 30 birds was thus studied.

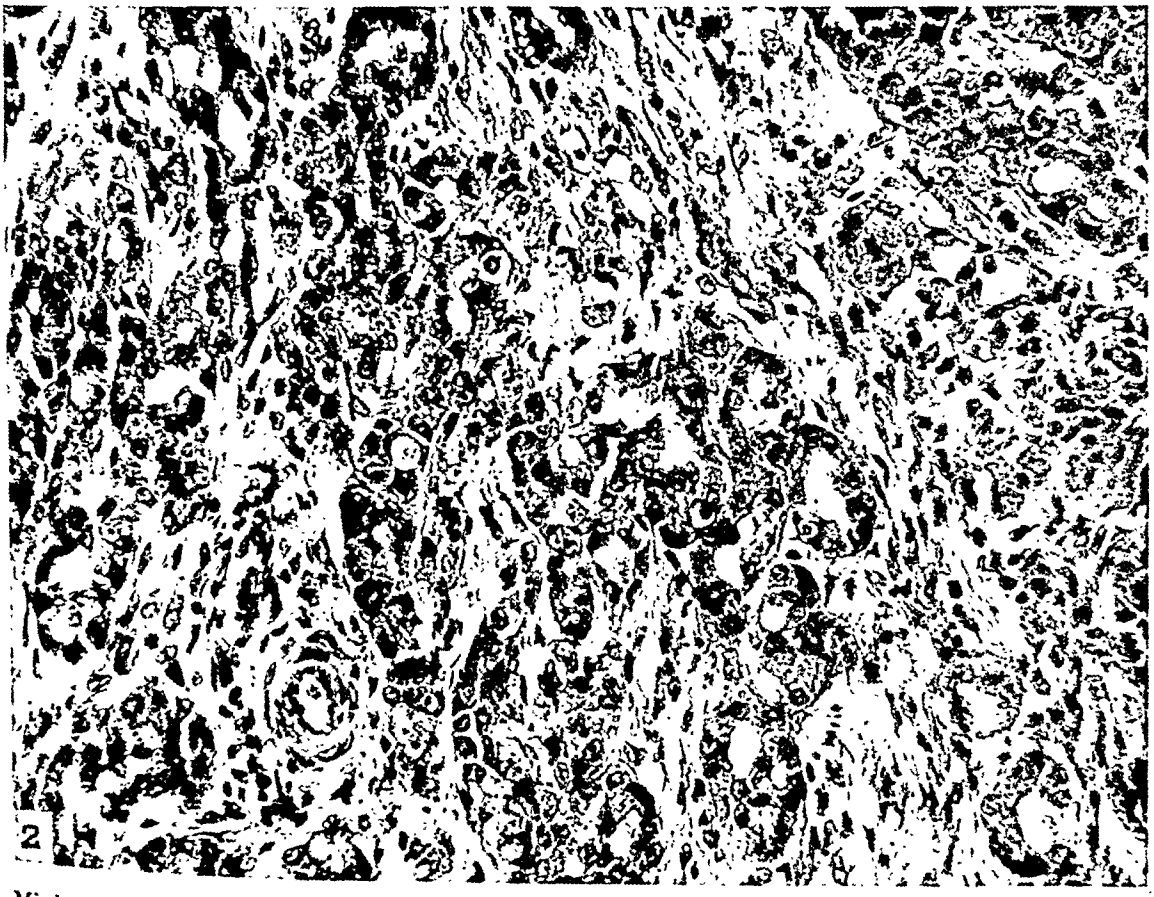
The majority of the ducklings took the diet voraciously. During the ensuing months many of the meat-fed birds developed marked edema of the extremities, periarticular fibrosis, arthritic changes and calcareous deposits about the joints. The bills and webs became white. Administration of carotene had no effect on this deficiency in pigmentation.

In none of the 30 animals that were depancreatized did a permanent hyperglycemia ensue. However, in most instances, a marked hyperglycemia was noted for 2 to 4 days after the operation, reaching levels as high as 900 mg. per cent in some birds. Following this, the blood-sugar level returned to normal or the animal died within 4 days with hyperglycemia.

Animals operated upon at the age of 6 to 7 months revealed grossly and microscopically normal pancreatic tissue. In animals that had been on the diet for periods longer than 7 months, the pancreas was small and difficult to remove. It felt cartilaginous, and cut like cartilage or like markedly fibrotic tissue. Histologic sections of these pancreases revealed varying degrees of fibrosis ranging from slight patchy change to almost complete replacement of normal structure by a hyperplastic connective tissue resembling a neoplasm. In the pancreases of nearly all ducks on the diet for longer periods (8 to 18 months) fibrosis predominated, whereas in those from animals on the diet for short periods (6 to 8 months) relatively little abnormality was noted. The normal pancreas of the duck is illustrated in Figure 1. Typical changes that occur in meat-fed ducks are depicted in Figures 2, 3 and 4.

* Received for publication, March 5, 1942.





Mirsky, Elgart, Nelson and Wasserman

Fibrosis of the Pancreas

It is obviously not feasible to attempt to explain the mechanism involved in the production of the change noted above, beyond noting that the diet of meat with cod liver oil was deficient in a large number of dietary essentials. It is quite possible that some food factor may be necessary for the maintenance of the normal pancreas, or it may be that a meat diet induces first a stimulation and subsequently an exhaustion with atrophy and fibrosis of the pancreatic acini. Further studies to elucidate the mechanism responsible for the above mentioned changes are in progress.

SUMMARY

Ducks maintained on a diet of meat and cod liver oil develop pancreatic fibrosis that apparently is progressive. The first changes are seen in about 8 months.

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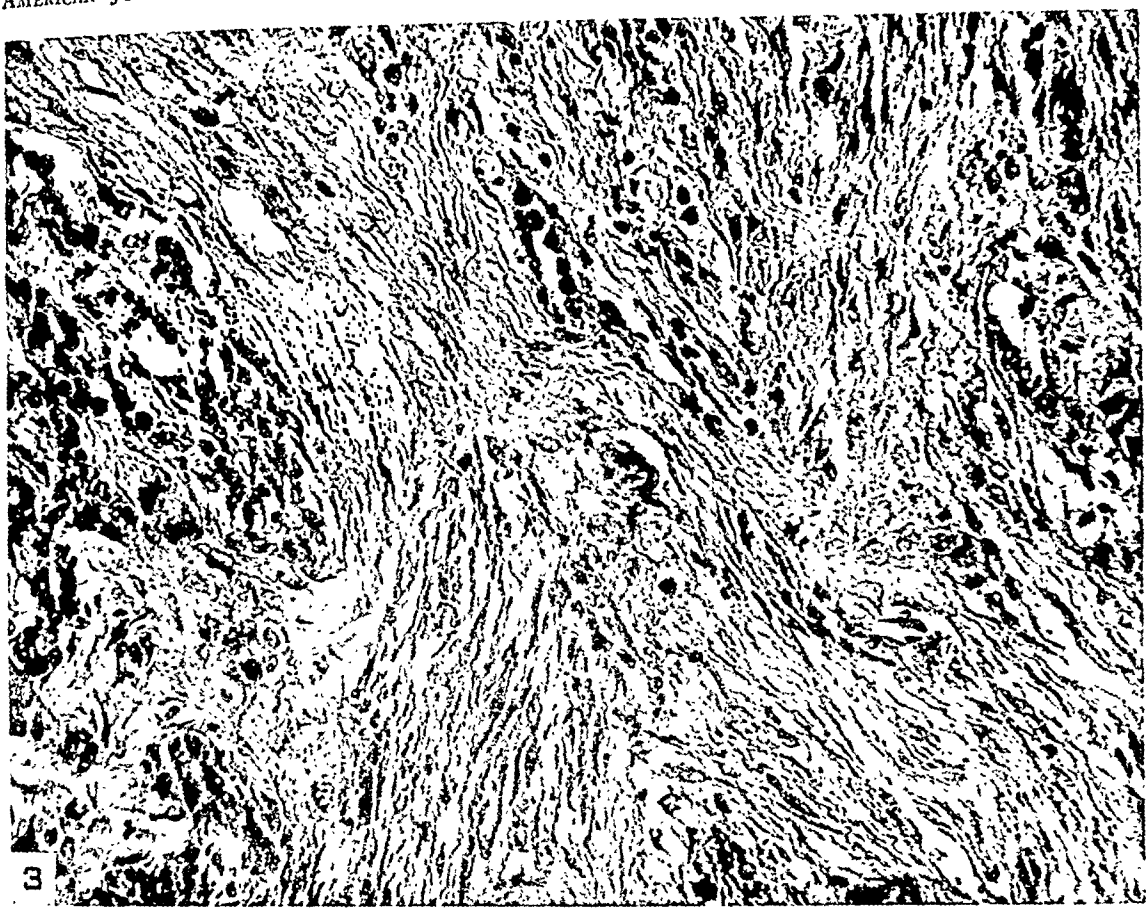
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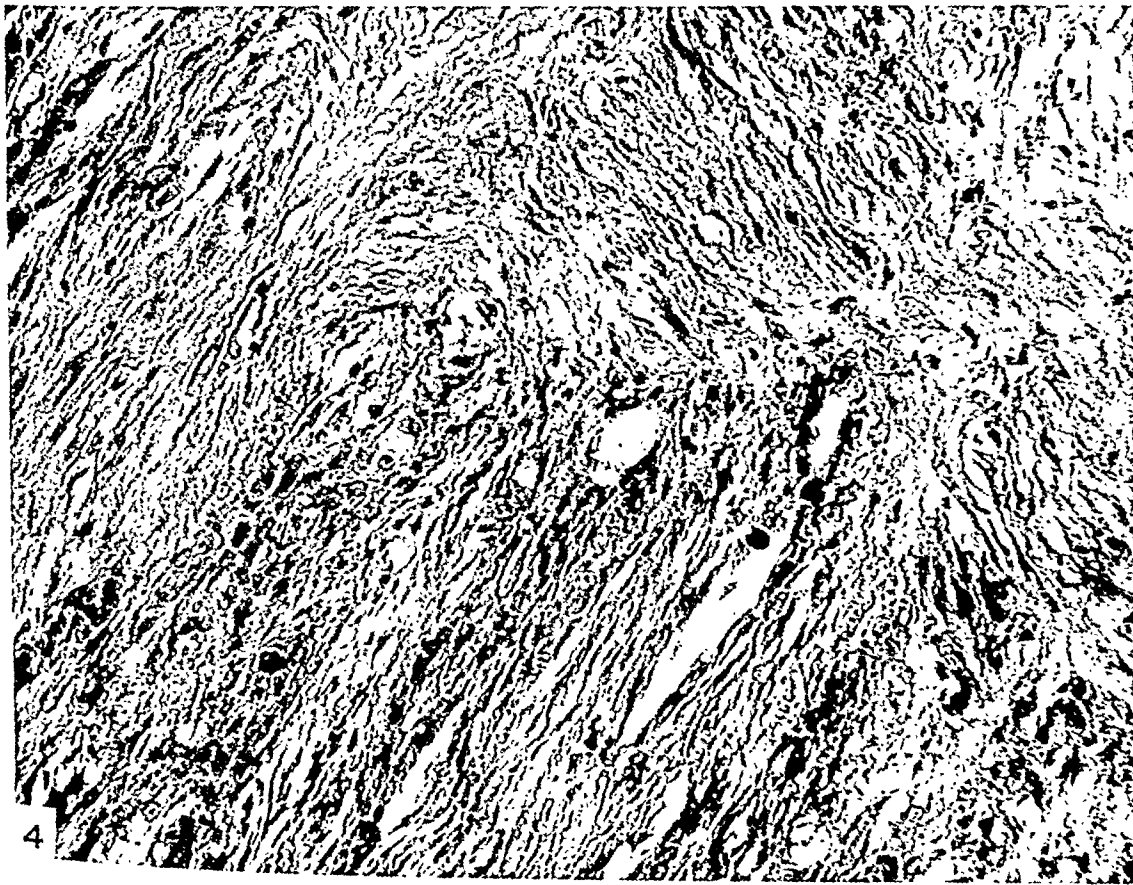
DESCRIPTION OF PLATES

PLATE 173

FIG. 1. Pancreas of a duck fed a normal herbivorous diet. Well preserved acinar tissue is seen. There is an occasional group of light-staining cells suggesting islet tissue.

FIG. 2. Pancreas of a duck fed meat for 6 months. Histologic detail is confused, the acini appearing somewhat irregular in size and shape. There is a distinct fibrosis with strands of connective tissue separating acini and somewhat distorting them. Some of the cells appear frayed and form enlarged glandular elements with papillalike structures projecting into the lumen.





Mirsky, Elgart, Nelson and Wasserman

Fibrosis of the Pancreas

PLATE 174

FIGS. 3 and 4. Pancreas of a duck fed meat for 16 months. There are areas where complete replacement fibrosis leaves no acinar or epithelial structures. The fibrosis is compact and relatively acellular. In isolated areas are found groups of epithelial cells in rounded nests surrounded by abundant fibrous tissue. The epithelium here is not in the acinar arrangement nor is it suggestive of islet tissue. There are areas which appear pseudoneoplastic in that the fibrous tissue presents a sweeping configuration in regions exhibiting the tendency to complete obliteration of the epithelial elements. No islet tissue is identifiable.

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all clinical complaints, and a 3-year follow-up found the patient entirely well and free from evidences of recurrence. In the other case (that represented by the specimen found in the Erdheim collection) no follow-up data are available. The follow-up histories in Codman's [3] nine cases showed all the patients well 3 to 10 years after coming under treatment.

Pathology

Gross Pathology

While we were able to examine pathologic material from all our cases, only in two did we have the opportunity of seeing the lesion intact in its setting. In one instance, the tumor was a rather small one in a femoral condyle and appeared as a small, friable mass of grayish tissue. In the other case,* the tumor was in the upper end of a humerus and involved the tubercles, metaphysis and an adjacent bit of the capital epiphysis. In this specimen, the articular cartilage of the humeral head was found preserved almost throughout. Along its posterior border it showed a chain of small marginal exostoses. In addition, on the dome of the head, there was a bumpy protrusion of the cartilage, caused (as was evident from subsequent sectioning) by a subchondral hemorrhagic cystic cavity in the tumor. Most of the articular cartilage over this bump was still intact, but posteriorly the cartilage was defective over a small area and a thin layer of connective tissue filled the base of the defect.

A coronal section through the specimen (Fig. 7) showed that while laterally the tumor affected the region of the tuberosities and extended into the capital epiphysis (to the articular cartilage) and into the metaphysis, medially parts of both the capital epiphysis and the metaphysis were uninvolved. In the cut section, the tumor measured 6 cm. in its long axis and 4 cm. across. The tumor tissue was sharply delimited from the neighboring uninvolved bone and the delimiting margin was convexly lobulated. Such tumor tissue as was not cystic or hemorrhagic (as it was in fully two-thirds of the lesion) was soft but rather firm and in some places even gritty and of a gray-yellow or gray-brown color. Nowhere in the tumor could one note grossly any tissue which could indubitably be regarded as bone. However, within the tumor and quite enclosed by tumor tissue but displaced from their normal position, one could note residual islands of epiphysial cartilage plate. The cystic and hemorrhagic areas were disposed mainly about the periphery. The cysts were 1 cm. or less in diameter and were outlined by a thin

* We found the specimen (catalogued as a sarcoma) in the collection of pathologic material preserved by the late Viennese pathologist, Jacob Erdheim, now housed in the laboratory of our hospital.

and is indeed likely to have been done before the diagnosis has been established on a histologic basis. In those of our cases in which this treatment was carried out, the lesion healed without recurrence, and function was restored. The end results in these cases were the same, whether or not the thorough curettement had been followed by moderate radiation therapy. On the other hand, in a case in which the lesion was in a femur and in which merely a punch biopsy was taken and the lesion subsequently treated with heavy irradiation, a contraction of the knee resulted, requiring arthrodesis for correction. Furthermore, in this case, islands of viable tumor tissue were still recognizable in the articular shavings removed in the course of the operative correction. It is true that Codman [3] cited two cases in which healing followed treatment by irradiation alone, but the significance of these cases is not clear, since the diagnosis was based solely upon the clinical findings and not even a punch biopsy specimen was taken. Altogether, we know that *thorough* curettage alone is sufficient to cure these lesions, and we see no necessity for using irradiation, either alone or as an adjuvant to curettage. On the whole, the weight of evidence seems to be against the necessity of irradiation and is certainly against very vigorous irradiation, either with or without previous curettage.

Follow-Up

In one case still under observation, only 9 months after an incomplete curettage of the lesion, the patient is entirely free of clinical complaints and the roentgenograms show substantial recalcification of the affected area. In another case, an incomplete removal was likewise followed by evidence of rapid calcification at the site of the lesion, and the clinical complaints disappeared within 5 months, but no further information on the subsequent history is available. In three cases the patients were discharged from the outpatient department as cured 1 year after a thorough curettage, since they no longer presented any complaints clinically and there was satisfactory ossification at the site of the lesion roentgenographically. None of these three patients has returned, and for two of them it is now 12 years since their treatment at the hospital was completed. Two cases in which, after a preliminary biopsy, the lesion was treated by heavy irradiation, have been followed for 5 years, and we know that in these there has been no recurrence of the lesion, though the neighboring joint (a knee in both instances) became stiff, requiring, as noted above, an arthrodesis in one case, because of contracture. In two instances the lesion was completely resected. In one of these, in which the lesion was a small one in the condyle of a femur, resection was followed by prompt disappearance of

philic appearance such as to suggest edema fluid or even chondromucin. When appropriately stained, the ground substance between the more loosely packed cells was found to contain interlacing collagen fibers in some places. However, what gave character to the histologic picture in these relatively cellular areas was the presence of spotty calcification (Figs. 8, 9 and 10). In some low-power fields, as many as two or three spots of calcification could be seen, some barely perceptible and others quite large and obvious. In sections of undecalcified tissue which had been stained with hematoxylin and eosin, the calcium appeared as bluish or purple granules. The calcium granules were not only deposited between the cells but were also found in the cytoplasm of the cells, especially where the focal deposition of calcium was relatively intense. The increased calcium deposition in such areas was associated with swelling of the tumor cells.

In surveying different fields of the tumor one could thus see clumps or nodules of tumor cells with large pale nuclei and purple-staining granules in their cytoplasm, surrounded by varying amounts of calcareous, intercellular, ground substance. In some fields, also, the focal calcium incrustation resulted in the appearance of calcareous blotches. When such an area was examined under high magnification it became evident that most of the cell nuclei within it were either disintegrating or had already disappeared, and the cellular architecture was sometimes found represented merely by faint outlines of the former cell walls. Thus, where the calcification had become more intense, focal areas of necrosis appeared in the tumor tissue. The devastation of the cells within the areas of intense calcification stood out very clearly in portions of the cellular tumor which had been decalcified and then stained with hematoxylin and eosin. Neighboring areas of focal calcification and necrosis sometimes spread and fused, leaving little if any intervening viable tumor tissue. Such areas represented the yellowish calcareous bits of gross material which can be found among the curettings from a benign chondroblastoma. Altogether, when one traces the evolution of the focal calcification, one cannot doubt that the necrosis of the tumor cells is secondary to the calcification and does not provoke it (Figs. 11 and 12).

Let us consider now the fate of the calcified and necrotic areas. About the periphery of these areas was seen a process of collagenization and the appearance of what seemed to be hyalinized connective tissue. This collagenized connective tissue progressively encroached upon and replaced the necrotic and calcified débris, roughly in the manner in which new bone is deposited upon dead bone through the process of creeping replacement. In the course of this encroachment the calcified

wall of connective tissue. Some of the hemorrhagic areas showed cystic softening and were apparently in the process of resolution into well defined cysts.

The appearance of the tumor in this case is in harmony with that of the resected humeral specimen studied by Phemister,[6] in which the tumor proper was a soft mass of mottled brown, blue and gray tissue. In our remaining seven cases, the tumor tissue as represented by the curetted material was in part reddish gray, rather cellular and quite vascular or even hemorrhagic in appearance, and in part more grayish and gritty and flecked with yellowish calcific material.

Microscopic Pathology

One can bring out details and gain a composite histologic picture of the lesion by processing the tissue in various ways. It is worth while to study not only material which has not been decalcified or which has been decalcified in nitric acid but also material which has been decalcified in Müller's fluid. Furthermore, appropriately processed tissue should be stained not only with hematoxylin and eosin but with von Kossa's stain, van Gieson's stain and Mallory's methylene blue stain, among others.

The basic cell of the tumor, as revealed in tissue fixed in formaldehyde and stained with hematoxylin and eosin, was generally polyhedral or round and of moderate size. Its nucleus was relatively large, usually 15 to 20 μ in its greatest diameter, round or oval and more or less centrally placed. On the whole, the chromatin material of the nucleus did not appear to be distributed in any special pattern. A nucleolus, in a fairly central location, was commonly seen. Mitotic division figures ordinarily had to be searched for, but in some areas they were more numerous. The cytoplasm and the delimiting membrane of the tumor cells failed to stand out prominently in some places, though in others they were quite evident. The general run of such cells was varied by some larger uninuclear cells, a few hyperchromatic cells and an occasional small giant cell containing two or three or even a few more nuclei. Here and there were also seen larger giant cells in varying numbers with many nuclei, usually appearing in small collections. However, as has already been stated and as will be shown more fully later, these giant cells were associated mainly with secondary changes in the tumor and hence are not significant in the sense of being of fundamental importance in its cytology.

In some fields the tumor cells were closely compacted, while in adjacent fields they were more loosely packed, being separated by varying amounts of interstitial material. The latter had a semifluid, baso-

tumor showed densification of the spongy trabeculae and fibrosis of the marrow. Where the tumor had approached the articular cartilage the latter was found to be thinned out and eroded or even completely destroyed. When the tumor was in the vicinity of the attachment of an articular capsule, the synovium in that region was likely to show villous hypertrophy and fibrous thickening in consequence of irritation.

Discussion

Our object is to show: (1) why the lesion in question does not represent a giant cell tumor; (2) why we interpret its basic tumor cells as cartilage germ cells; and (3) why we think the lesion should not, however, be regarded as an ordinary benign chondroma and also not as a chondrosarcoma or osteogenic sarcoma.

The question of the correctness of the common interpretation of the lesion as a giant cell tumor of bone (albeit a variant) logically brings up the question of the definition of giant cell tumor. We [1] interpret the latter as a genuine neoplasm arising from the undifferentiated supporting connective tissue of the marrow and composed of a more or less vascularized network of spindle-shaped or ovoid stromal cells, heavily, though not necessarily evenly, interspersed with multinuclear giant cells which are part of the tumor tissue itself. Furthermore, we have found that a giant cell tumor of bone shows but little collagenous differentiation of its stroma, no evidence of calcification and only rarely any evidence at all of ossification.

Certainly, benign chondroblastoma of bone does not meet these cytol ɔgic criteria. In the first place, in relatively unmodified areas of the t umor, the basic tumor cells are polyhedral in shape and closely packed or interspersed with a small amount of basophilic intercellular mate ial which suggests edema fluid or chondromucin. All through such areas one can note spots and patches of calcification of this interstitial mate ial and even of the local tumor cells proper. In such cellular and relati ely well preserved areas of the tumor, large multinuclear giant cells are very sparse or altogether lacking, and thus clearly do not constitute an essential element in the pattern of the lesion. Those few giant cells that are found in such areas usually have only a small number of nuclei and apparently represent multinuclear tumor cells derived by fusion of uninuclear polyhedral cells. As already noted, it is only in those areas of a benign chondroblastoma in which there has been a considerable amount of necrosis and hemorrhage, or around those areas showing chondroid organization, that one may find many giant cells. Furthermore, these giant cells show a patchy or clumped arrangement, are larger than the tumor giant cells and may be regarded as representing multinuclear macrophages.

material was confined more and more closely to the center of the area undergoing replacement and was steadily diminishing in amount. Finally, there were large patches of collagenous hyaline or chondroid tissue in place of the necrotic tissue. These areas were separated by others in which viable noncalcified polyhedral tumor cells were still present.

In the hyaline chondroid material were seen viable cells widely separated from each other within lacunae and surrounded by a ground substance which, when stained with eosin, showed an acidophilic tendency, apparently on account of its collagen content. The hyaline chondroid tissue manifested progressive organization, amounting, in some small areas, to transformation into more typically cartilaginous tissue. In rare places there was also seen deposition of calcium in the matrix of this organized chondroid material and what appeared to be islands of bone apparently formed through direct metaplasia (Figs. 13, 14 and 15).

In the description of the gross appearance of the lesion of the humerus which we had the opportunity of studying intact in its setting, it was noted that the tumor presented also areas of gross hemorrhage, softening and small cysts resulting from the organization of hemorrhage. Microscopically, it was seen that there were a good many areas of free hemorrhage in the tumor tissue and also numerous vascular spaces and even sinuses. The hemorrhages were in the vicinity of calcified necrotic areas or even within noncalcified tumor tissue. Indeed, vascular spaces and sinuses were lined throughout or in part by tumor cells proper or were bordered by wide zones of rather hyalinized connective tissue.

We come now to the multinuclear giant cells. There were indeed, as already noted, occasional small giant cells (with two or three or even several more nuclei) distributed among the polyhedral tumor cells. These giant cells were probably actual tumor giant cells formed by fusion of the smaller uninuclear cells (Fig. 8). However, about the periphery of necrotic areas undergoing hyaline and chondroid metaplasia, and especially in the vicinity of large vascular sinuses and free hemorrhage, numerous large multinuclear giant cells were seen, often in aggregates or clumps. It is the presence of many giant cells in such areas that has been the basis in the past for conceiving this lesion to be a variant of giant cell tumor. These giant cells undoubtedly represent multinuclear macrophages such as are commonly found in any skeletal lesion in the vicinity of local hemorrhage, organization, fibrosis, chondrification, or ossification (Fig. 16).

Sections of the osseous tissue beyond the advancing margin of the

the special physical properties of firmness, elasticity and pliability, and its special chemical contents are chondroalbuminoid, chondromucoid and chondroitin-sulfuric acid, plus collagen. Furthermore, such cartilage tissue, which is mainly the so-called hyaline cartilage, tends to show basophilism of its ground substance, and (as a feature preliminary to its degeneration and replacement) swelling of the cells and calcification of its ground substance.

If we project these features of cartilage tissue against the tissue of the tumor we are discussing, we find indications which favor an interpretation of this tumor as having much of the nature of cartilage about it. Thus, the basic cells of the tumor produce a basophilic ground substance containing collagen fibers which surround and embed the cells. Wherever the tumor tissue is calcifying, its cells swell and become separated by even more of the ground substance, which becomes impregnated with calcium. The only way in which the tissue of this tumor differs from true hyaline cartilage is that swelling of its cells and calcification of its matrix are not precursors to replacement by osseous tissue through the process of endochondral ossification. However, a hyaline chondroid tissue does replace the calcified and necrotic tumor tissue and may even undergo spotty ossification by the process of direct metaplasia. Thus there is a very definite aura of cartilage about the whole tumor.

Nevertheless, it would be incorrect to interpret this tumor simply as a chondroma of bone. If it were an ordinary chondroma, even a modified one, its full-fledged cartilaginous nature would be obvious both grossly and microscopically. Central chondromas of bone are composed of facets or mosaics of mature, well differentiated cartilage cells, between which there is likely to be, at least in many parts of the lesion, a richly hyaline matrix. Even in areas in which the intercellular matrix is meager or has undergone myxoid or mucoid degeneration, the cells and the tissue as a whole still retain the character of actual cartilage. None of this is true in regard to benign chondroblastoma. Hence, the tumor cells of this lesion, which do not mature into full-fledged cartilage cells, should, in our opinion, be interpreted as chondroblasts, and the tumor itself as a chondroblastoma, the word "benign" being prefixed to indicate that the lesion is not a malignant one, in spite of its richly cellular character.

To refute the other idea—that the lesion is a chondrogenic or osteogenic sarcoma—we would need only to refer to the uniformity with which the lesion has been found benign by those who have studied it closely. In addition, a chondrosarcoma not only develops out of full-fledged cartilage but shows cellular, and particularly nuclear, atypism

It might be argued at this point that it is precisely because of the cytologic differences which have been noted between benign chondroblastoma and the regulation giant cell tumor that the former should be called a variant * of the latter. However, if one does so regard it, one is placing a stronger emphasis upon superficial resemblances than upon fundamental practical differences, for these two lesions are not only different cytologically but also in respect to their clinical manifestations and prognosis. Specifically, in contrast to benign chondroblastoma, the regulation giant cell tumor is generally a rather large lesion, is seen in older subjects (20 years of age or more), does not present histologic or roentgenographic evidence of calcification and is often an aggressive lesion, frequently recurring, even becoming malignant, and requiring wide excision or amputation of the affected part.

We must now answer the question of why we are interpreting the tumor specifically as a "benign chondroblastoma" and its cells as cartilage germ cells. First, it may be useful to define cartilage tissue. Various histologists are by no means in accord on this definition.[17] Discarding academic disputes, one can say that cartilage tissue in the strictest sense is tissue whose cells produce an interstitial substance or matrix which embeds these cells and contains fibers. This matrix has

* Altogether, a whole theoretical superstructure of so-called "giant cell tumor variants" has been uncritically built up, encasing other lesions also. Thus we[8] have found no evidence to support the idea, fathered by Mönckeberg,[9] developed by Konjetzny[10] and adopted also by Geschickter and Copeland,[11] that solitary bone cyst represents a healing form of giant cell tumor. Also, we cannot sustain Ewing's[4] conception of "certain benign circumscribed spindle-cell myxosarcomas with few or no giant cells" as representing a form of giant cell tumor, and we believe that most of these represent, instead, solitary expressions of the disorder which we[12] have described under the head of "fibrous dysplasia of bone." The lesion which has been held (by Kolodny,[13] for instance) to represent the "xanthic" variant of giant cell tumor is not, to our mind, a giant cell tumor either, but rather a spindle cell fibroma of bone (nonosteogenic fibroma[14]) merely having a sprinkling of giant cells, often containing hemosiderin-bearing phagocytes and sometimes showing areas having groups of lipophages. Examples of this same lesion without the presence of lipophages are also described (by Geschickter and Copeland,[15] for instance) as examples of "giant cell variants of the bone cyst in the metaphyseal ends of the long bones" (p. 268) and the "giant cell variant of osteitis fibrosa" (p. 300, Fig. 195). Furthermore, osteofibromas (osteogenic fibromas) of bone, when appearing elsewhere than in the skull and particularly the jaw bones, have also been referred to (for instance, by Geschickter and Copeland[15]) as the "spindle cell variant" of giant cell tumor (p. 339, Fig. 219). Finally, we feel that the resemblance between the so-called "brown tumor" of hyperparathyroidism and certain epulides, on the one hand, and genuine giant cell tumor, on the other hand, is merely a very superficial cytologic one, and that the pathogenetic differences between the "brown tumor" and giant cell tumor are very much more important.[16]

In summary, we point out our doubts as to the propriety of forcing into the category of giant cell tumor, albeit as variants, certain bone lesions which bear merely a superficial resemblance to it. These supposed variants have been classified as variants of giant cell tumor mainly because they contain larger or smaller numbers of multinuclear giant cells, rather than on the basis of their total histologic structure. It is not only theoretically inaccurate but practically disadvantageous to force into the category of giant cell tumor, lesions which are fundamentally different from it in histologic appearance and prognosis.

it is not proper to regard this lesion as a variant of giant cell tumor and hold the tumor cells to be chondroblasts and the tumor a benign chondroblastoma.

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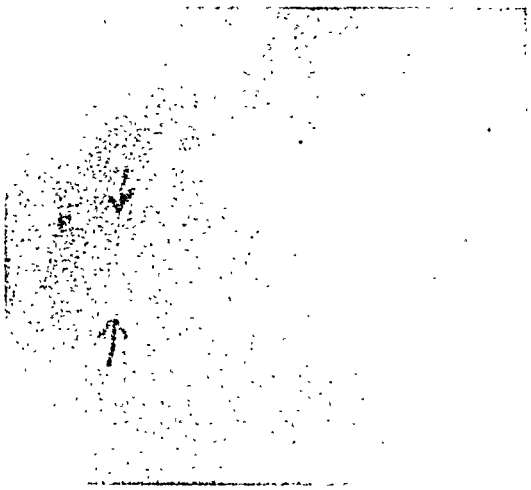
of this cartilage, such as one does not see in chondroblastoma. Specifically, a chondrosarcoma shows numerous binuclear cartilage cells with plump nuclei, large or giant cartilage cells with single or multiple nuclei, and, if it is full-blown, more than a sprinkling of mitotic division figures. Cytologically, there should also be no confusion with osteogenic sarcoma. The latter develops out of bone-forming mesenchyme, and, in contrast to benign chondroblastoma, not only may produce a considerable amount of clear-cut cartilage by proliferation, but always lays down some tumor osteoid tissue and bone directly between the tumor cells proper.

Summary and Conclusions

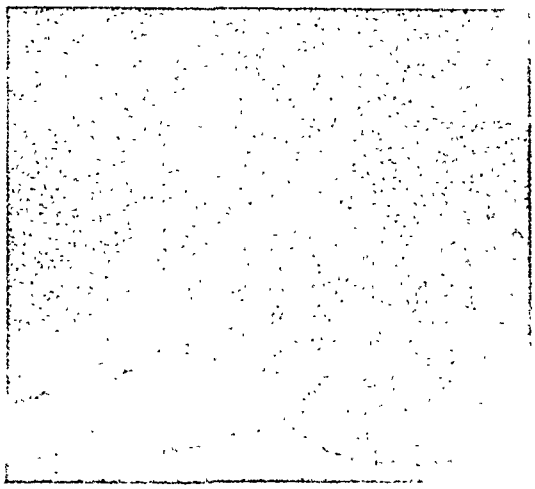
The lesion which we have described and called benign chondroblastoma of bone has usually been conceived in the past as a variant of giant cell tumor (calcifying or chondromatous) and sometimes even as a malignant tumor of bone and specifically a chondrosarcoma or an osteogenic sarcoma. The tumor starts its development in an epiphysis, usually of some long bone. It may extend to the articular surface of the epiphysis and even into the metaphysis, but nevertheless rarely attains a size of more than 3 to 5 cm. in its largest diameter. We did not find that the lesion exhibits a predilection for the upper end of the humerus, although some previous workers have thought that it did. It does seem to occur particularly in males, and the patients are almost always adolescents or postadolescents at the time when they seek medical care for the condition. When the roentgenogram is considered in the light of the pathology of the lesion, there is much about it to help make the diagnosis on a clinical basis.

The basic tumor cell of the lesion is polyhedral or round and of moderate size, with a fairly large nucleus. The tumor cells may be closely packed or more loosely agglomerated, but what is very distinctive of the pathology is the presence of focal areas of calcification of the cellular tumor tissue. Wherever the calcification becomes intense, the tumor cells swell and undergo necrosis. The necrotic tumor tissue comes to be replaced by hyaline chondroid tissue which subsequently may show spots of ossification. There may be areas of hemorrhage, and one may see large vascular sinuses bordered by viable tumor tissue, necrotic tumor tissue, or hyaline chondroid material which has replaced the necrotic tumor tissue. Clumps of large multinuclear giant cells may be seen in the areas of hemorrhage, about the vascular sinuses and even in the hyaline chondroid tissue.

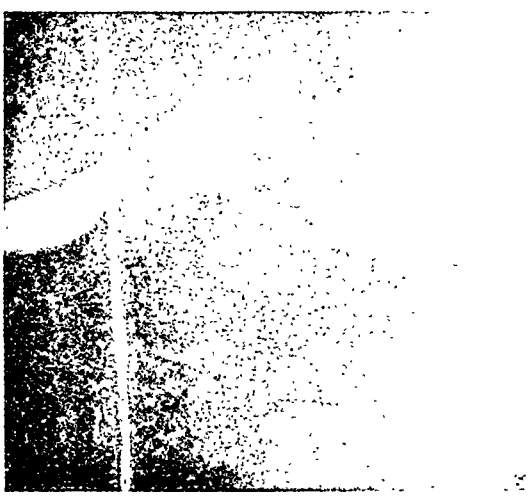
The lesion is entirely benign and heals without recurrence after thorough curettage, even without supplementary radiation. We think



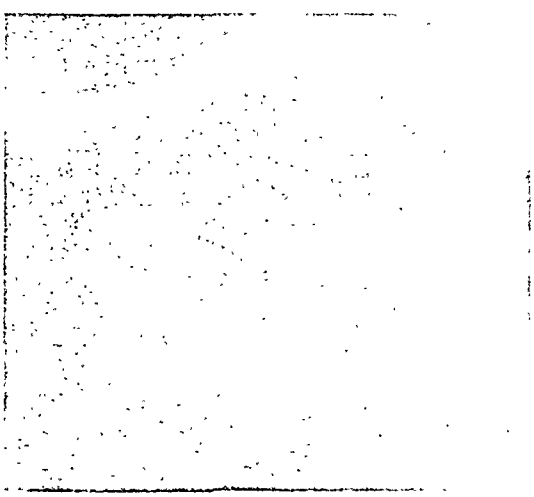
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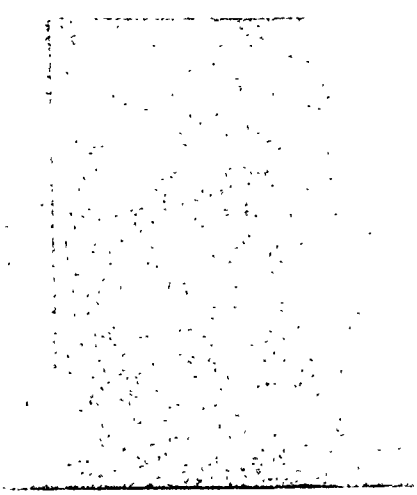
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3



4



5



6

Jaffe and Lichtenstein

Benign Chondroblastoma of Bone

DESCRIPTION OF PLATES

PLATE 150

FIG. 1. Roentgenograph showing a lesion 1.5 cm. in diameter, located in, and limited to, the lower epiphysis of a femur and, specifically, to the external condyle. Radio-opacity may be seen, indicating sclerosis of the bone beyond the limits of the lesion. The patient was a boy of 13 years who complained of swelling, tenderness and weakness of the knee, walked with a slight limp and presented some local heat and muscle atrophy. There was no antecedent history of injury.

FIG. 2. Roentgenograph showing a lesion 4.5 cm. in its greatest diameter, located in the lower epiphysis of a femur, specifically in the intercondylar region, and also involving the metaphysis. There is a radio-opaque line more or less outlining the lesion. The latter casts a rather vague, fuzzy shadow which, however, yields some suggestion of trabeculation. The patient was a boy of 15 years who, for 1 year, had been having mild difficulties relating to the knee. There was a history of an injury (fall) 2 years previously, but the relevance of this is not clear.

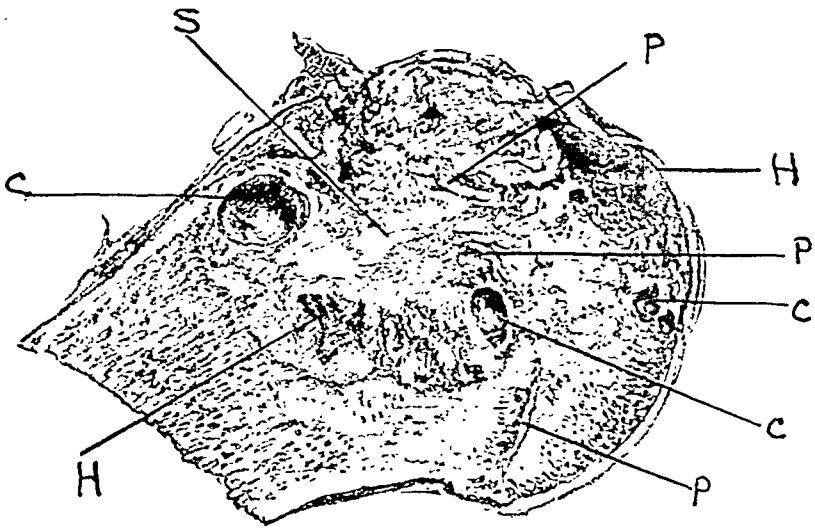
FIG. 3. Roentgenograph showing a lesion 3 cm. in its greatest diameter, located in the upper epiphysis of a tibia and extending from the epiphysial cartilage plate to the intercondyloid eminence, where it has actually perforated into the joint. There is a fuzzy, mottled shadow cast by the lesion, and the surrounding bone is radio-opaque. The patient was a boy of 14 years who complained of difficulty with his knee (stiffness, swelling, limp) for 3 months before admission to the hospital. His history also included the statement that he fell from a bicycle about 1 month before admission, but this fall could not be clearly related to the present bone disease.

FIG. 4. Roentgenograph showing a lesion 5 cm. in its greatest diameter, located in the proximal epiphysis of the tibia (involving parts of both condyles) and also extending into the adjacent metaphysis. The lesion appears to be vaguely mottled, with a delimiting line about it. The patient was a boy of 17 years who had been complaining for about 6 months of slight swelling of the knee (especially on walking), some local heat and tenderness, and some limitation of flexion. His history included the statement that 7 months prior to admission he had tripped and injured his knee.

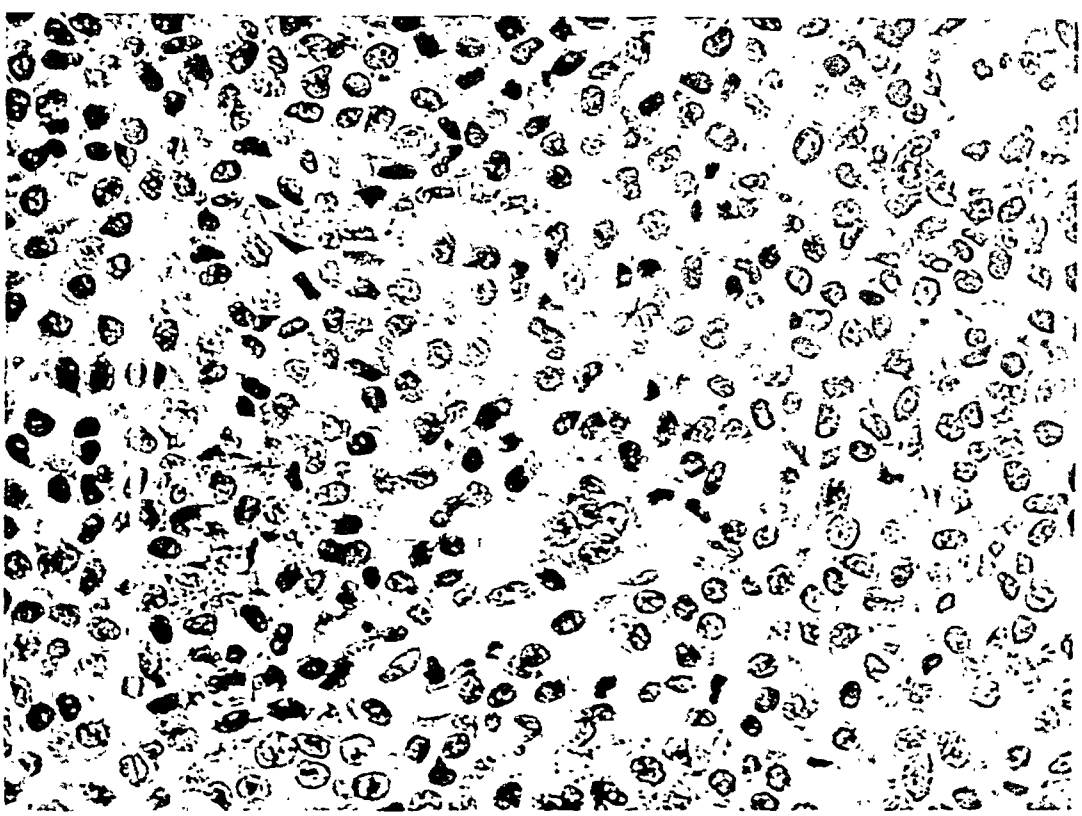
FIG. 5. Roentgenograph showing a lesion 4 cm. in its greatest diameter, located in the lower epiphysis and metaphysis of a tibia. In this view the x-ray shadow cast by the lesion is rather vague. The patient was a boy of 15 years who complained mainly of some pain in the ankle, of several months' duration.

FIG. 6. Lateral view of the lesion shown in Figure 5, indicating more precisely that the lesion is situated posteriorly and has bulged out the cortex of the tibia. In this view the lesion shows considerable trabeculation.

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8



Jaffe and Lichtenstein Benign Chondroblastoma of Bone

PLATE 151

FIG. 7. Drawing (actual size) of coronally cut upper end of a humerus showing a benign chondroblastoma involving the tuberosity and adjacent portions of the capital epiphysis and metaphysis. The specimen came from a boy of 16 years and was obtained by surgical resection done on the assumption that the lesion was a sarcoma. It may be seen that the tumor extends to the articular cartilage of the head and that this cartilage actually bulges a little where the head is involved. The tumor tissue proper was a whitish gray-brown in color. C = cysts, H = areas of hemorrhage, P = bits of epiphysial cartilage plate and S = a large area of chondroid scar tissue.

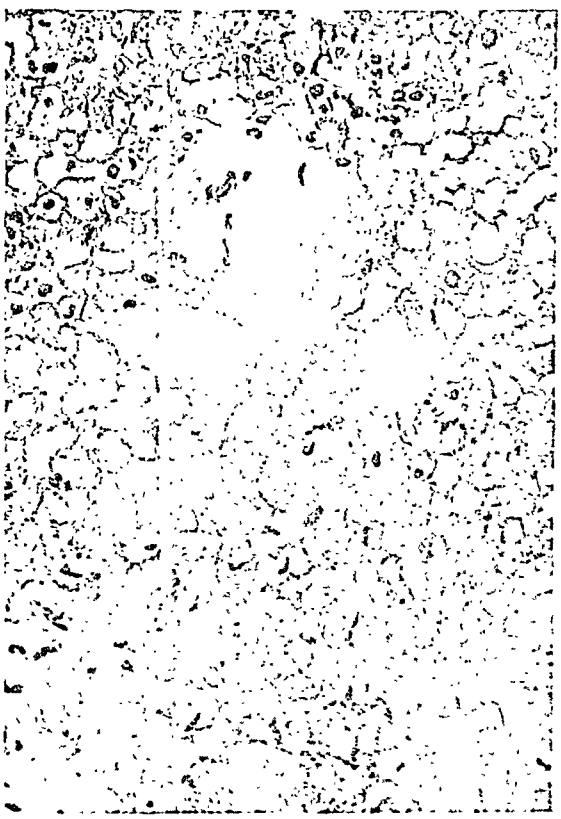
FIG. 8. Photomicrograph showing the general cytologic pattern in a cellular and relatively unmodified area of a lesion. The stippling toward the left of the picture represents beginning calcification between some of the cells. Toward the center there is beginning swelling of the cells and a small multinuclear giant cell. × 450.



9



10



11



12

Jaffe and Lichtenstein Benign Chondroblastoma of Bone

PLATE 152

FIG. 9. Photomicrograph showing the general cytologic pattern in a cellular area in which the deposition of intercellular material and the process of calcification have advanced farther than in Figure 8. × 450.

FIG. 10. Photomicrograph showing a nidus of even more densely calcified tumor tissue surrounded by tumor tissue not yet so modified. The tissue from which the slide was prepared had not been treated with nitric acid before being embedded. The grittiness of such a calcareous area (and, *a fortiori,* of a more calcareous one) is likely to result in fragmentation when the slides are prepared from paraffin-embedded tissue, so that satisfactory photographs of intensely calcified areas are difficult to obtain. × 230.

FIG. 11. Photomicrograph showing an area in which the calcification had originally been more intense than that shown in Figure 10, but decalcified with nitric acid before embedding. Pale shadows of necrotic cells may be seen where the calcification has been particularly intense. × 230.

FIG. 12. Photomicrograph showing intermingled viable and necrotic (and previously heavily calcified) tumor tissue with areas of cystic softening. The tissue from which this slide was prepared had been treated with nitric acid. × 100.

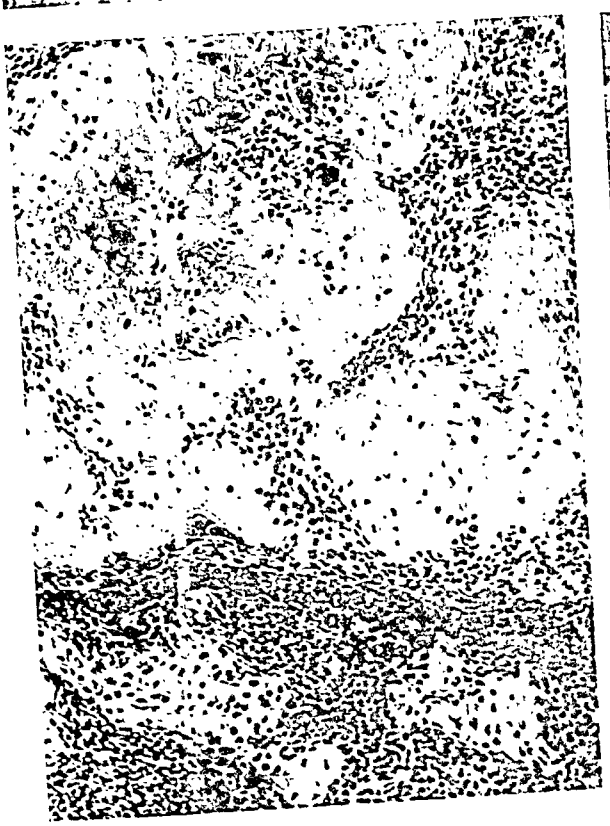
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15



16



Jaffe and Lichtenstein

Benign Chondroblastoma of Bone

PLATE 153

FIG. 13. Photomicrograph of tissue which, after being decalcified in Müller's fluid, has been stained with hematoxylin and eosin. The dark blotches represent areas of necrotic and still calcareous tumor tissue, between and about which collagenization and chondroid metaplasia has been taking place. × 53.

FIG. 14. Photomicrograph showing further progress in the process of collagenization and chondral replacement of a necrotic area. Some residual necrotic and calcified tissue is seen, surrounded by chondral material. × 72.

FIG. 15. Photomicrograph showing chondroid areas intermingled with cellular tumor areas. Here and there in the chondroid tissue secondary calcification is apparent. In some lesions, the calcifying chondroid material may undergo spotty osseous metaplasia. × 72.

FIG. 16. Photomicrograph showing an area of cellular tumor in which there has been blood leakage, the hemorrhagic area being outlined by multinuclear giant cells. × 220.

CARCINOID TUMORS OF THE RECTUM DERIVED FROM ERSPAMER'S PRE-ENTEROCHROME CELLS *

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Although a great deal has been written about carcinoid tumors, there exists very little information about their occurrence in the large bowel. In the Laboratory of Surgical Pathology of Columbia University there are records of four cases of reputed carcinoid of the rectum, and in the neighboring Laboratory of Pathology are two other rectal cases found among 13,809 routine autopsies. There are no examples of carcinoid tumors in the cecum or colon. Table I shows the distribution of all the cases involving the large bowel known to me. A study of the Columbia University cases has shown that the carcinoid tumor in the rectum sometimes displays certain morphological features which differ from the commoner carcinoids found in the appendix and small intestine. These variations seem worth recording because it is not easy to recognize these tumors as carcinoids unless one is familiar with them. I had been uncertain about the nature of case 1 for 9 years, and no one to whom I submitted it was able to help me. Finally, a review of the more recent histological investigations of the basigranular cells of the gastrointestinal tract afforded a reasonable explanation of the vagaries shown by the tumors reported in this group and made it possible to trace such seemingly dissimilar neoplasms, as are described in cases 1 and 2, to a common ancestor.

REPORTS OF CASES

Case 1

C. L., a married American negress, 19 years of age, came to the Proctology Clinic of the Presbyterian Hospital in January, 1932, suffering with bleeding from the rectum and increasing constipation of 1 year's duration. Examination showed a rounded polypoid mass, 2 cm. in diameter, situated anteriorly in the lower rectal segment and attached to the first valve, about 5 cm. from the anus on the left side. The mass was movable, without infiltration at the base. Biopsy was done on January 8, 1932, and on January 26, 1932, a wedge-shaped segment of the rectal wall, including the tumor, was excised. In September of the same year a simple adenomatous polyp was removed from near the site of excision. The patient has been followed continuously since and remains without any evidence of local disease. She was last seen on May 21, 1941. She had enlarged right cervical nodes believed to be due to tuberculosis and enlarged tonsils. Otherwise, physical examination was negative.

Gross Examination. The specimen consisted of a segment of rectal wall about 2 cm. long and a submucosal tumor, 18 by 17 by 15 mm.

* Received for publication, March 2, 1942.

most parts, so that it was difficult to determine the outlines of most of them. Where this was seen the nucleus generally occupied an eccentric position. The nucleus was quite large, with sharply defined chromatin (Fig. 2). No mitotic figures were observed. The cytoplasm was very finely granular and in general stained a neutral tint. The most striking and characteristic feature was the presence of slightly larger fuchsinophil granules found in the cytoplasm at one side of the nucleus in a very considerable number of the cells. These granules were best seen when Masson's aniline bluè-ponceau-acid fuchsin stain was used. They were not differentially stained with eosin and after 96 hours in ammoniacal silver nitrate they showed only the very faintest browning. In this same preparation the granules of the basigranular cells of the crypts of Lieberkühn were jet-black. In the section stained by Masson's trichrome method there were a number of basigranular cells in the crypts of Lieberkühn with fuchsinophil granules (Fig. 2), but not nearly as many as had silver blackened granules after treatment with ammoniacal silver nitrate.

The degenerated cells generally showed pyknotic nuclei and had cytoplasmic vacuoles which were reddened with scharlach R. Lipoid-filled vacuoles were occasionally found in well preserved cells. The tumor cells nowhere formed acini, rosettes or pseudorosettes. They did not secrete mucin or colloid.

Case 2

L. S. was a British West Indies negress, 41 years of age. Her trouble began 4 months before admission with diarrhea and pain in the left lower quadrant of 5 days' duration followed by constipation, anorexia, nausea, loss of 9 lbs. in weight and persistence of the pain. Proctological examination showed a 5 mm. sessile tumor situated 5 cm. from the mucocutaneous junction on the left anterior wall of the rectum. On October 4, 1938, the tumor was locally excised. It was later found that her symptoms were due to regional enteritis and on April 3, 1939, an ileocolectomy was done at another hospital. Examination did not show any other carcinoid tumors. She was known to be alive on October 19, 1940.

Gross Examination. The specimen was received in Bouin's fluid. It consisted of an intramucosal, seemingly circumscribed, tumor nodule, measuring 7 by 4 mm. and surrounded by a thin collar of mucosa between 1 and 2 mm. wide.

Microscopical Examination. The tumor lay chiefly in the submucosa but extended through the muscularis mucosae into the overlying mucosa which was much thinned and atrophic. The tumor had a very striking appearance, being composed of cords of slender columnar and prismatic cells so arranged that the long axis of the cells was at right angles to the long axis of the cord. The cells were in a single layer and the cords were bent and curled so as to resemble heaps of ribbon carelessly coiled

(Fig. 1). The tumor was covered with elevated mucosa to which it was attached in places. An intact collar of mucosa varying from 2 to 7 mm. in width surrounded it and deep to it were the intact muscle coats of the rectum. The cut surface of the tumor was firm and appeared pearl

TABLE I
Carcinoid Tumors of the Large Bowel

Author	Anatomical distribution		
	Cecum	Colon	Rectum
Ariel	0	1	0
Brunschwig	0	0	1
Corten	1*	0	0
Feyrter	0	5	0
Humphreys	0	0	1
Kaufmann	0	1	0
Koch	0	0	1*
Mallory	0	0	1
Martin	0	1	0
Mayo and Wilson	1*	0	0
Raiford	1*	1*	0
Saltykow	0	1	1
Siburg	0	0	1*
Stout	0	0	6
Wyatt	1*	0	0
Total	4	10	12

* Indicates malignant carcinoids.

white, faintly tinged with yellow, and mottled with red due to extravasated blood. The specimen was fixed in Bouin's fluid.

Microscopical Examination. The tumor consisted of large, solidly packed masses of cells loosely held together by a delicate supportive framework of widely spaced reticulin fibers bearing infrequent blood vessels (Fig. 1). This was easily seen with the Laidlaw silver reticulin stain but was very inconspicuous with Masson's trichrome stain and still more so after hematoxylin and eosin. At its periphery the tumor cells broke up into more definite strands. In places they lay just beneath the muscularis mucosae and sometimes passed through it to appear in the stroma surrounding the crypts of Lieberkühn. In three different places the crypts had disappeared and the tumor had reached the surface. The tumor cells nowhere appeared in direct continuity with the cells of the crypts. Extravasated blood was found in several parts of the tumor and many tumor cells showed pyknosis but there was no extensive necrosis. In the depths, the tumor remained within the confines of the submucosa and at no point penetrated the inner muscle coat.

The individual tumor cells were large, irregularly rounded and occasionally columnar or prismatic, with poorly defined cell membranes in

the twisted cords of columnar cells sometimes united abruptly with solid masses of polygonal tumor cells which resemble those of ordinary carcinoids. No tumor cell mitoses were seen, although there were many in the mucous cells of the crypts. The tumor cell cytoplasm contained many tiny granules but these were not fuchsinophil and they were unaffected by long immersion in ammoniacal silver nitrate. In the crypts of the overlying mucosa were many basigranular cells which were strongly fuchsinophil but only a few cells had granules darkened by ammoniacal silver nitrate. The tumor lay chiefly in the submucosa but separated the fibers of the muscularis mucosae, and strands of tumor cells were found between the crypts. Some of these had been destroyed and in others the deeper portion of the crypt was composed of tumor cells. The sections did not show the relation of the deep surface of the tumor to the rectal wall. No tubules, rosettes, or pseudorosettes were formed. No mucin or colloid had been secreted. Some cells contained empty vacuoles. Lipoid stains were not done.

Case 4

Mrs. S. L. was an American Jewess, 38 years of age. Two months before operation proctoscopic examination showed a small, firm nodule on the anterior wall of the rectal ampulla. In 2 months' time this increased to a diameter of 5 mm. There was rectal bleeding 2 days before operation, ascribed to hemorrhoids which were present. A barium enema showed no other abnormalities of the large bowel. The nodule was excised with a fulgurating snare on November 13, 1939, and the base was cauterized with the actual cautery. There has been no evidence of recurrence since that time. (The case has been made available by Dr. Alvin O. Severance of San Antonio, Texas, who recognized it as a carcinoid tumor. Several other pathologists, including two with national reputations whose experience with tumors is very large, had called it carcinoma.)

Gross Examination. When received by Dr. Severance the specimen measured 6 by 7 by 4 mm. and was covered on one surface with apparently intact mucosa. The nodule was fixed in formaldehyde.

Microscopical Examination. Like the three preceding cases this tumor lay in the submucosa and extended between the layers of the muscularis mucosae where its strands were found in some places replacing the crypts, elsewhere between the crypts and in one instance united with the cells of a single crypt. The strands of tumor cells were predominantly columnar or prismatic, with rounded, ovate, or cylindrical nuclei showing no mitoses and situated usually toward one pole. The cytoplasm was partly granular and partly amorphous but the granules were not fuchsinophil nor were they affected by ammoniacal silver nitrate after immersion for 72 hours. In several areas, however, instead of ribbons of coiled columnar cells arranged in festoons, there were polygonal cells crowded together in large solid masses as in case 1.

at haphazard (Fig. 3). The nuclei were elongated to fit the narrow shape of the cells and were generally oriented toward one pole. The chromatin was arranged as in the nuclei of case 1, and did not have the large prominent nuclei characterizing the mucous cells of the crypts of Lieberkühn. Slender strands of dense fibrous tissue separated the cords, and thicker strands separated groups of cords. In one place a tumor cord united with cells forming a crypt of Lieberkühn in such a way that the bottom of the crypt was formed of tumor cells (Fig. 4). A search through 42 sections showed only a few mitotic figures. These were found in tumor cells in the mucosa close to the goblet cells of the crypts in which mitosis was very active. The cytoplasm contained a few diffusely scattered granules which were faintly acidophilic with eosin and fuchsin but which were never strongly acidophilic as in case 1, and were not blackened by ammoniacal silver nitrate even after 96 hours. In this same preparation the granules of the basigranular cells in the crypts of Lieberkühn were clearly defined and a dark brown. No attempt to form rosettes or pseudorosettes was observed nor was any mucin or colloid formed. A few empty, basally situated intracellular vacuoles were seen. A scharlach R stain showed that these contained lipoid. No tubules were formed, except in a few instances where cords of tumor cells were found in the mucosa. The tumor seemingly had not infiltrated deeply, as an uninvolved layer of submucosa lay deep to it.

Case 3

L. H. was an American negress, 34 years of age. She complained of soreness and anal protrusion after stool, which began 4 years before admission, and had recently grown worse, with bleeding. By proctological examination, hemorrhoids were found as well as a firm, sessile, rounded mass about 1 cm. in diameter on the anterior rectal wall about 5 cm. above the anal margin. It had a mottled, yellow appearance. It was excised with a surrounding collar of mucosa on November 7, 1941. When histological examination seemed to show that excision was incomplete, a second operation was done 11 days later and the rectal wall in the region of the scar was excised through an abdominal approach. No tumor was found in this specimen. The patient was well when examined 2 months later.

Gross Examination. The tumor measured 11 by 8 mm. and had some ragged tags of mucosa attached to it. It had a yellowish gray tinge and was sharply defined. The cut surface was also grayish. It was fixed in Zenker's fluid with 5 per cent glacial acetic acid added.

Microscopical Examination. This tumor resembled that of case 2, with certain differences (Fig. 3). It was composed of strands and ribbons of columnar and prismatic cells arranged in festoons, but the cells were shorter and slightly plumper so that the nuclei tended to be rounded. The cells tended to shrink away from the supporting fibrous stroma so that they appeared to lie in spaces. As shown in Figure 3,

polygonal cells. Occasionally there are palisade cells next to the connective tissue outlining a cell column and rarely there are columnar and prismatic cells grouped around a cavity or vesicle which may contain a tiny albuminous droplet of colloid aspect. This description is in accord with the appearance of the appendiceal and ileal carcinoids in this laboratory, but it does not fit the appearance either of case 1, with its huge solid masses of polygonal cells, or the ribbonlike festoons of columnar and prismatic cells seen in cases 2, 3, 4 and 6. Such festoons are occasionally found in pancreatic islet adenomas as illustrated by Laidlaw (1938). Polygonal cells are present in these four tumors but they are infrequent and the striking appearance of the festoons makes it easy to overlook them. Like ordinary carcinoids, these tumors have cells with lipoid-filled vacuoles, but none shows the formation of vesicles or rosettes and none secretes albuminous material. All of them involve the mucosa and in cases 2, 3 and 4, tumor cells are found in contact with and forming part of crypts. Occasionally in the mucosa, tumor cells surround open spaces, but this arrangement is never found elsewhere. Whether the involvement of the crypts is primary or secondary is not apparent.

The reactions of the intracellular granules is also at variance with those of appendiceal and ileal carcinoids. Silvering was done in all but case 6. Case 5 will not be considered, both because it was an autopsy case and also because the technical procedure was not carried out in this laboratory. Cases 1 and 2 were fixed in Bouin's fluid, case 3 in Zenker's fluid with 5 per cent glacial acetic acid added, and case 4 in formaldehyde. In every case, Fontana's ammoniacal silver nitrate was used according to Masson's formula. In the first three cases the same preparation showed blackening or browning of the granules of the basigranular cells in the crypts but had no effect on the tumor cell granules of cases 2, 3 and 4, and caused only the very faintest browning in case 1. In case 1, the intracellular granules were strongly fuchsinophil, in case 2 they were weakly fuchsinophil, while in the other cases they did not seem to be selectively different from the rest of the cytoplasmic content. In all of the five cases, Masson's aniline blue-ponceau-acid fuchsin reddened the granules of the basigranular cells of the crypts.

At this point one naturally inquires: Are these unusual formations really carcinoid tumors derived from the basigranular cells of the mucosal crypts or are they some other kind of tumor, such as, perhaps, the islet cell adenoma to which they have certain resemblances? A heterotopia of islet cells in the rectum has not been described so far as I am aware. Therefore, unless the basigranular cells themselves are islet cells, one does not need to give this explanation serious consideration.

In the overlying crypts a few basigranular cells with fuchsinophil granules could be identified with difficulty, but none with browned or blackened granules were noted after silvering. No tubules, rosettes, or pseudorosettes had been formed and no mucin or colloid was secreted. Fat stains were not done.

Case 5 *

J. K. was a white American female, 42 years of age, who died from infection following perforation of the esophagus.

Gross Examination. At autopsy a firm, white, oval plaque, 7 mm. in diameter, was found in the submucosa of the anterior rectal wall. The specimen was fixed in Zenker's fluid.

Microscopical Examination. Because of postmortem changes, examination was not satisfactory. Like the other tumors, this was limited to the submucosa and infiltrated the mucosa. It was made up of widely separated strands of polygonal cells, smaller than those of the four preceding tumors and more nearly like those in the carcinoids of the appendix and small intestine. The cytoplasm contained granules which were not affected by fuchsin or ammoniacal silver nitrate. No tubules, rosettes, or pseudorosettes were formed and no mucin or colloid was seen. Fat stains were not done. In spite of postmortem changes in the epithelia of the crypts, basigranular cells with fuchsinophil granules were seen. They were unaffected by silver nitrate.

Case 6

J. B. was a colored American male, 43 years of age, who died following the attempted removal of a leiomyosarcoma of the stomach.

Gross Examination. At autopsy there was found an elevated, yellowish, slightly flattened, freely movable, firm nodule in the mucosa and submucosa of the rectum just above the anus. The specimen was fixed in Zenker's fluid.

Microscopical Examination. This tumor also lay in the submucosa, separated the fibers of the muscularis mucosae and ramified in the mucosa where the crypts had been eroded and in some places destroyed by it. It did not extend beneath the submucosa. Like case 3, it was made up chiefly of festoons of short cylindrical cells with hardly any tendency to form polygonal cells, which were seen in only a few places. No tubules, rosettes, or pseudorosettes were found. No special stains were done.

Discussion

Masson's (1928) description of the carcinoids of the appendix and small intestine stated that they consist chiefly of cords of round or

* Cases 5 and 6 come from the Laboratory of Pathology of Columbia University and are reported by permission of Dr. James W. Jobling, Director.

first, which he called clear nonargentophil pre-enterochrome cells, there are no argentophil or argentaffin granules at all. The second stage he called pre-enterochrome acidophil argentophil cells. These contain acidophil granules which can take up silver, but as they contain no enteramin they do not reduce it and become blackened nor do they give the diazo reaction nor fluoresce. Finally, he recognized what he called empty enterochrome cells which have granules that have been emptied of enteramin. Vialli and Erspamer (1933) supposed that the specific substance in the granules (enteramin) is probably ortho-diphenol that has an unknown and perhaps not very complex side chain with a free area in the ortho-position. Jacobson (1939) found that the granules contain both a pterine and a carbohydrate.

Vialli and Erspamer first noted the acidophil pre-enterochrome cells in the large bowel of the dog and cat. It seems to be true that both types are found there also in humans.

The speculations regarding the function of the basigranular cells are interesting chiefly because they are so much at variance with each other. Masson and Berger suggested that the substance secreted by the cells is emptied directly into nerves; Erös (1933) and Jacobson (1939) believed that the secretion plays a part in erythropoiesis; Popoff (1939) thought that the granular cells have no definite function at all, but are simply stages in the rejuvenation of functionally exhausted and refractory mucous cells. Reference to Patzelt's review shows that other ideas have also been expressed such as: That the granules are an exocrine secretion, that they have a resorptive function, that they are concerned with digestion, that they are related to suprarenal medullary cells, to the oxyphil cells of the hypophysis and to the islet cells of the pancreas. It will suffice to say that none of these hypotheses stands as proved. The nature and meaning of the basigranular cells of the gastrointestinal tract remain unknown.

However, the investigations which have been carried out on these cells are of great aid in interpreting the appearance of this present group of tumors of the rectum. If the basigranular cells are formed from the goblet cells and only secondarily assume their pyramidal or wedge shape and acquire their granules, it is not so difficult to explain the variants seen in the tumor morphology. The cords or ribbons of columnar and prismatic cells represented in Figures 2 and 3 may be homologues of the basigranular cells in an earlier phase of metamorphosis, while the polygonal cells more nearly approximate the appearance of these cells as they appear in the crypts (Fig. 2). If we may believe the work of Clara and of Erspamer that the granules of the basigranular cells are ordinarily associated with a material which

No case of tumor of the large bowel has given symptoms of insulin secretion: no one, including myself, has been able to demonstrate the specific staining reactions of islet cell granules in carcinoid tumors, nor, on the other hand, will islet cell granules blacken with ammoniacal silver nitrate. Therefore it seems safe to conclude that, in spite of a morphological resemblance, neither the basigranular cells nor carcinoid tumors are derived from islet cells. There remains for consideration the relationship of these rectal tumors to the basigranular cells. In order to answer this question I reviewed the information extant pertaining to these well known but mysterious cells.

In addition to Masson, there are a number of histologists and pathologists who have devoted much time and thought to the investigation of these cells. Among these should be noted especially Clara, Erspamer, Feyrter, Jacobson, Patzelt and Popoff. The work up to 1936 is extensively reviewed by Patzelt in Möllendorff's Handbuch. For the purposes of identification and of limiting these remarks to the large bowel and rectum, it may be noted that the basigranular cells are scattered at intervals among the mucus-secreting cells of the crypts of Lieberkühn. They are usually pyramidal cells with their bases resting upon the basement membrane and their apices usually not reaching as far as the lumina of the crypts. These cells have nuclei elevated above the basally situated nuclei of their neighboring cells and in the basal cytoplasm are found a considerable number of fine granules (Fig. 2). These cells have been called by a variety of terms, such as yellow cells, argentaffin or chromo-argentaffin; chromophil, chromaffin or enterochromaffin, and basigranular cells, and by the names of Nicolas, Kultschitzky, Schmidt and Ciaccio. Of all these the purely descriptive term "basigranular" seems preferable because it is neither a proper name nor a questionable attribute. Clara (1934) has shown that these cells are derived in the human embryo from undifferentiated intestinal epithelium, and Popoff (1939) believed that they continue to be formed during adult life from the mucus-secreting cells. There is some indirect support for this viewpoint in the observations of Cordier (1924) and Siburg (1929), both of whom described carcinoid tumors in which mucus-secreting cells were associated with the granular cells.

The granules have naturally excited a great deal of interest and speculation. Erspamer (1939) designated the cells as enterochromaffin and recognized four variants. The typical enterochromaffin cells have granules containing a chemical substance which he called enteramin. This makes them give a positive diazo reaction, become blackened by ammoniacal silver nitrate and show fluorescence in ultraviolet light. These typical cells are preceded by two different earlier phases. In the

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enables them to blacken ammoniacal silver nitrate, while in some instances, particularly in the large bowel, the granules may lack this substance and so be unable to blacken ammoniacal silver nitrate, an adequate explanation is furnished for the observation that in cases 1, 2 and 3 basigranular cells of the rectal mucosa were darkened by silver, while the granules in the tumor cells of the same preparations were not. It is necessary to be specific about fixatives and the preparation of silver used because, as Hamperl (1932) has pointed out, whether or not silver will blacken granules depends upon these two factors. Some silver preparations, such as that of Gros-Schultze,* will blacken not only melanin and intestinal basigranular cells but also islet cells, suprarenal medulla cells and anterior hypophysis cells. In order to complete this rather complicated picture, the reaction with ammoniacal silver nitrate also depends upon the presence or absence of enteramin in the granules.

Information regarding other carcinoid tumors of the large bowel is exceedingly scanty. The reports indicate that ammoniacal silver nitrate was tried in only seven cases. It succeeded in four (Brunschwig; Wyatt; Raiford, 2 cases) and failed in three (Feyrter; Martin; Siburg). It was impossible to learn whether the basigranular cells of the overlying crypts were blackened in these last three cases.

It would seem, then, that the festooning ribbons of columnar cells represent the reproduction on a large scale of the basigranular cells in an early phase of differentiation when they approximate the shape of columnar crypt cells. The failure of the tumor cell granules to blacken ammoniacal silver nitrate after proper fixation and in the same preparations in which the granules in the basigranular cells in the crypts were blackened can be explained if one supposes that the latter contained enteramin while the tumor cell granules lacked it. If one accepts this, these tumors can be regarded as carcinoids composed of Erspamer's pre-enterochrome cells.

Summary

Six probable carcinoid tumors of the rectum are described. Five of them differ cytologically from the common carcinoids found in the appendix and ileum. It is suggested that these are carcinoid tumors composed of Erspamer's pre-enterochrome cells.

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DESCRIPTION OF PLATES

PLATE 154

FIG. 1. Case 1. The gross pictures in the upper right portion of the field show the external appearance of the excised specimen with the rectal mucosa elevated by the tumor, and the mottled surface of the nodular growth on cross section. The low-power photomicrograph made from a complete section through the entire tumor shows its situation in the submucosa, its invasion, and in two places its complete erosion of the mucosa, and, below, its failure to invade the muscular coats. It also shows a division of the tumor cells into huge, solid masses separated by slender fibrous bands. An enlargement of some of the cells forming one of these masses is inset.

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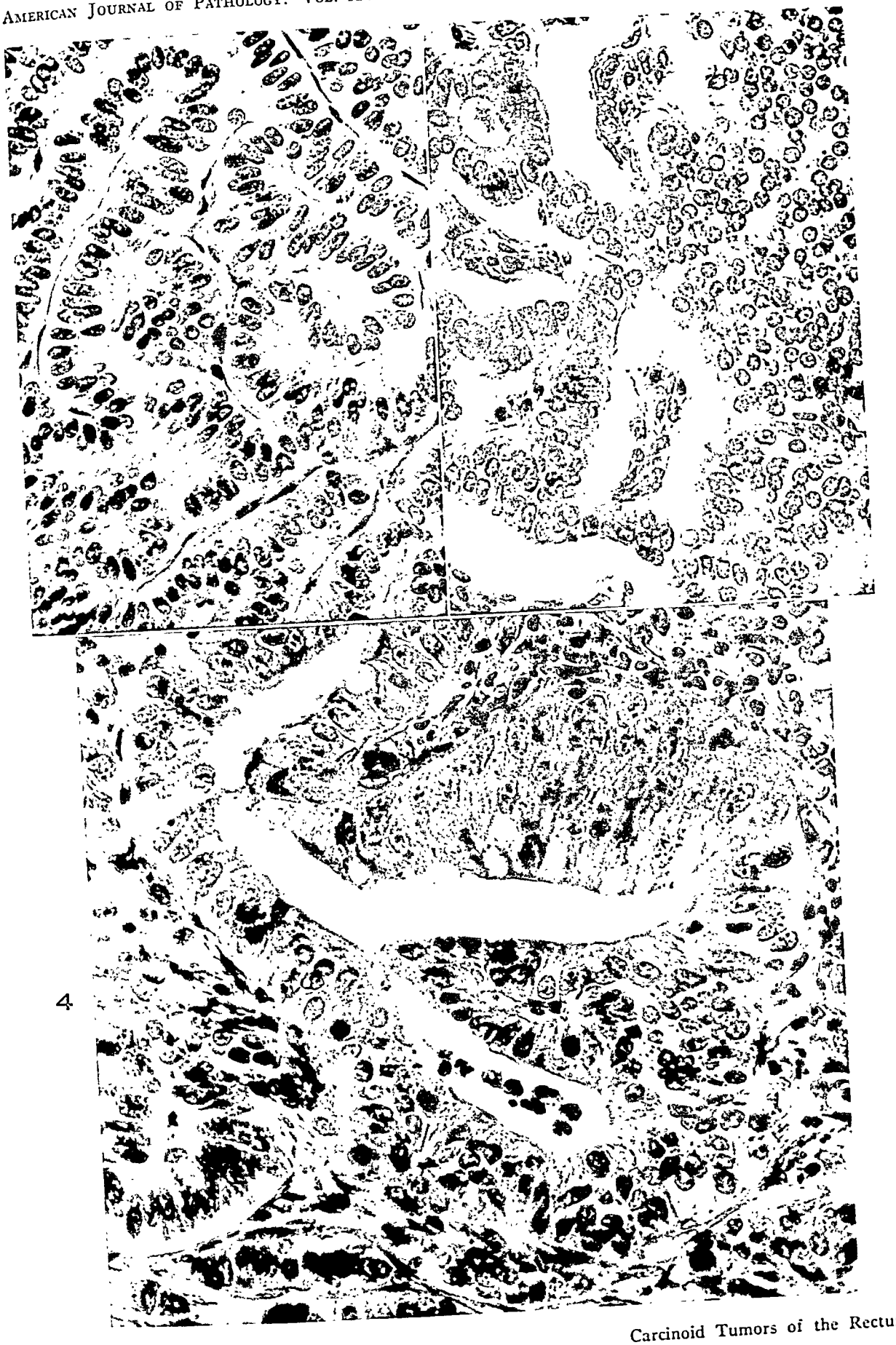
2

Carcinoid Tumors of the Rectum

Stout

PLATE 155

FIG. 2. A composite picture, slightly reduced from several photomicrographs. In the top row are three different basigranular cells in crypts of Lieberkühn from the rectum. They show the pyramidal or wedge shape of the cells, the elevated rounded nucleus differing from the cylindrical nuclei of the adjacent goblet cells, the granules in the basal pole and the lighter appearance of the cytoplasm surrounding the granules. In the middle row are characteristic polygonal cells from case 1 with groups of darkened granules eccentrically placed and only partly filling the cytoplasm. In the lower row at the left are columnar and prismatic tumor cells from case 1. One of these shows granules at one pole. This cord of cells was in the mucosa away from the large solid masses. At the lower right are columnar and polygonal cells from an appendiceal carcinoid tumor. Two of them show granules in one pole. All sections were stained with Masson's aniline blue-ponceau-acid fuchsin stain. In every instance the dark granules were stained a deep red in contrast to the other neutrally tinted cytoplasmic granules. × 1350.



3

4

Carcinoid Tumors of the Rectum

Stout

PLATE 156

FIG. 3. Case 2 (left); case 3 (right). The cells of case 2 are columnar and prismatic, arranged in ribbonlike sheets which twist and turn in every direction. The sheets are one cell wide and are separated by delicate reticulin fibers, sometimes bearing capillaries. In case 3 the ribbons have shrunk away from the fibrous framework, sometimes producing the appearance of tube formation. A point of junction is shown between ribbons of columnar cells and a mass of polygonal cells. Hematoxylin and eosin stain. × 416.

FIG. 4. Case 2. A distorted crypt of Lieberkühn lined by columnar goblet cells above toward the lumen of the colon, and by columnar tumor cells below in the direction of the submucosa. Masson's trichrome stain. × 416.

THE RENAL TUBULE (NEPHRON) AS AFFECTED BY MERCURY*

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Pictures and descriptions of renal tubular lesions have not been adequately specific with regard to the part of the tubule affected. Lack of specificity is understandable in view of the difficulties involved in ascertaining the precise locus and extent of such lesions. Indeed, this study would not have been undertaken without the compelling stimulus of convincing evidence of segmental structure and function of the tubule as revealed in various experiments and observations. Since among metallic poisons, mercury was accredited by Suzuki [1] with inducing a lesion in the terminal third of the proximal convolution, it seemed important to determine in requisite detail the fixed or variable site or sites of such tubular lesions as could be definitely attributed to the action of mercury.

This study was completed several years ago but owing to unavoidable circumstances the results obtained have thus far been published only in abstracts.[2,3] It would appear that segmental injury to the tubule is additional evidence of its structural and functional differentiation—especially functional—since the lesion caused by the action of mercury is restricted almost exclusively to portions of the proximal convolution which, in most vertebrates whose kidneys have been studied, have not been shown to be composed of histologically distinct segments. An aspect of such damage suggested the approximately simultaneous injection with the mercury of certain solutions in order to discover whether the site or degree of injury was changed thereby.

The data presented refer primarily to tubular damage induced by the action of mercuric chloride when it is injected into certain mammals and the frog. In another paper, functional changes relative to this damage will be discussed.

MATERIAL AND METHODS

The kidneys upon which the effects of mercury were studied were those of man, the rabbit, guinea pig and frog. Pieces of each kidney were fixed in Bouin's fluid and prepared for histological study while other portions of the same kidney were macerated in dilute HCl. Macerated pieces were dissected with glass needles under a microscope and each proximal convolution, as well as other portions of the tubule (in fortunate instances, the entire tubule) was towed to an area of the dish used in dissection where it was uncoiled as far as possible and

* Received for publication, January 22, 1942.

and daily thereafter for 5 days (no. 1 only) and similarly 4 cc. of sodium thiosulfate with each injection of mercuric chloride (no. 3 only). No. 2 was given only the two injections of mercuric chloride. No. 3A, on the 1st, 3rd, 5th, 14th and 15th days, was given 2, 2, 3, 3 and 3 mg. of mercuric chloride with 3 cc. of Ringer's solution simultaneously on each of these 5 days. No. 4, on the 1st and 14th days, was given 1.6 and 1.2 mg. of mercuric chloride, respectively, with 4 cc. of Ringer's solution on these days and on the 3rd and 5th days also. Nos. 5, 6 and 7 were given one injection of 1.6 mg. of mercuric chloride with 4 cc. of sodium ferrocyanide (no. 5), with 4 cc. of sodium thiosulfate (no. 6) and 4 cc. of sodium ferrocyanide daily for 3 days (no. 7).

Human

No.	Weight in Kg.	1	2	3	4	5	6	7	8	Died, hours
4911	59	T	T	T	T	T	T	T	..	36
392292	61	P	P	P	P	T	T	T	..	360

P = partial; T = total.

No. 392292 was given several injections of sodium thiosulfate and 50 per cent glucose solution.

Rats

No.	Weight in gm.	1	2	3	4	5	6	7	8	Died, killed, hours
45	225	..	..	..	..	T	T	..	..	48 k
50	200	..	..	..	T	T	T	..	..	118 d
70	250	..	..	..	..	T	T	..	..	115 d
75	200	..	..	..	..	T	T	..	..	105 d
78	228	..	T	T	T	T	T	T	..	124 d
83	294	..	..	T	T	T	T	T	..	78 d
93	87	..	..	..	..	S	S	..	..	22 d
94	265	..	T	T	T	T	T	..	..	48 k
95	258	..	..	T	T	T	T	..	..	48 k
96	271	..	..	T	T	T	T	..	..	48 k

S = slight; T = total.

Rats nos. 45 to 96 were given 0.8 mg. of mercuric chloride only.

No.	Weight in gm.	1	2	3	4	5	6	7	8	Died, killed, hours
92	214	..	..	T	T	T	T	..	..	72 k
97	332	P	P	P	P	T	T	..	..	72 k
98	400	..	..	P	P	T	T	..	..	72 k
99	330	..	..	P	P	T	T	..	..	72 k

P = partial; T = total.

Rats nos. 92 to 99 were given one injection of 0.8 mg. of mercuric chloride with 3 cc. of urea simultaneously and 3 cc. daily thereafter for 3 days.

attached to a coating previously applied to the bottom of the dish. Damaged segments of tubules so isolated are detectable and measurable owing to a collapse of their walls or by their marked fragility. Histological study of similar tubules in the sectioned pieces supplemented the study of those which were isolated. Thus, by a combination of microdissection and histological study, the extent and degree of injury were determined.

The solutions used, expressed in grams per 100 cc., were as follows: mercuric chloride, 0.04; sodium ferrocyanide, 1; sodium thiosulfate, 1; ferric ammonium citrate, 1; urea, 20. The mammals were injected intraperitoneally and the frogs in the dorsal lymph sac.

Results of Experiments

Table I

Varying Degree of Necrosis in Segments of Proximal Convolutions, Tabulated by Eighths

Rabbits

No.	Weight in Kg.	1	2	3	4	5	6	7	8	Died, killed, hours
1	4.4	..	..	..	..	..	T	T	..	384 d
2	3.3	..	..	..	..	..	T	T	..	3192 d
4	3.0	..	..	..	..	..	T	T	..	3216 k
5	4.1	..	..	..	T	T	T	T	..	88 d
6	3.4	..	..	..	..	..	T	T	..	360 d
9	4.1	T	T	T	T	T	T	T	..	162 d

T = total.

Rabbit no. 1 was given on the 1st, 3rd, 5th and 11th days, 2, 4, 5 and 5 mg. of mercuric chloride simultaneously with 5, 10, 12 and 12 cc. of Ringer's solution; likewise, nos. 2 and 4, except that sodium ferrocyanide and sodium thiosulfate, respectively, were injected instead of Ringer's solution. No. 5 was given one injection of 10 mg. of mercuric chloride with 25 cc. of sodium ferrocyanide and no. 6, on the 1st and 14th days, was given 10 mg. of mercuric chloride simultaneously with 25 cc. of sodium thiosulfate. No. 9 was given 10 mg. of mercuric chloride only.

Guinea Pigs

No.	Weight in Kg.	1	2	3	4	5	6	7	8	Died, killed, hours
1	0.57	S	S	S	S	S	S	S	S	171 d
2	0.58	S	S	S	S	S	S	S	S	175 d
3	0.57	S	S	S	S	S	T	T	T	51 d
3A	0.61	S	S	S	S	S	T	T	T	285 d
4	0.51	..	..	..	..	..	..	..	..	452 d
5	0.46	T	T	T	T	T	T	T	T	30 d
6	0.52	P	P	P	T	T	T	T	T	40 d
7	0.37	..	..	P	P	T	T	T	T	304 d

P = partial; S = slight; T = total.

Guinea pigs nos. 1, 2 and 3, on the 1st and 3rd days, were given 1.6 and 1.2 mg. of mercuric chloride, respectively, with 4 cc. of sodium ferrocyanide simultaneously

No.	Weight in gm.	1	2	3	4	5	6	7	8	Died, killed, hours
79	229	T	T	T	T	T	..	..	..	70 d
81	292	T	T	T	T	T	..	..	..	54 d

T = total.

Rats nos. 79 and 81 were given one injection of 0.8 mg. of mercuric chloride with 3 cc. of a mixture of equal parts of sodium ferrocyanide and sodium thiosulfate simultaneously and daily thereafter for 2 days.

Frogs

No.	Weight in gm.	1	2	3	4	5	6	7	8	Died, killed, hours
61	35	..	..	..	..	..	..	P	P	350 d
62	30	P	T	T	T	T	T	T	..	118 d
67	31	T	T	T	T	T	T	T	T	396 d
69	45	..	T	T	T	T	T	T	T	105 d
75	30	..	T	T	T	T	T	T	T	48 k
77	35	T	T	T	T	T	T	T	T	48 k
78	37	T	T	T	T	T	T	..	..	41 d

P = partial; T = total.

Frogs nos. 61 to 78 were given one injection of 0.6 mg. of mercuric chloride. No. 67 was given a like amount on the 7th day and no. 61 on the 7th and 10th days after the 1st injection.

No.	Weight in gm.	1	2	3	4	5	6	7	8	Died, killed, hours
79	26	T	T	T	T	T	T	T	T	62 k
80	25	T	T	T	T	T	T	T	P	60 d

P = partial; T = total.

Frogs nos. 79 and 80 were given one injection of 0.4 mg. of mercuric chloride with .2 cc. of Ringer's solution simultaneously and 2 cc. of it daily thereafter for 2 days.

No.	Weight in gm.	1	2	3	4	5	6	7	8	Died, killed, hours
81	25	P	P	P	P	S	S	S	S	28 k
82	25	T	T	T	T	T	T	T	T	38 k
83	30	P	P	T	T	T	T	P	..	51 k
84	25	T	T	T	T	T	T	T	T	51 k

P = partial; S = slight; T = total.

Frogs nos. 81 to 84 were given one injection of 0.4 mg. of mercuric chloride with 2 cc. of urea simultaneously and (nos. 83 and 84) 1 cc. of urea 24 hours later.

No.	Weight in gm.	1	2	3	4	5	6	7	8	Died, killed, hours
64	200	P	P	T	T	T	T	..	..	77 d
86	307	..	..	T	T	T	T	..	..	71 d
102	316	T	T	T	T	T	T	..	..	49 d
103	368	P	P	T	T	T	T	..	..	52 d
105	342	P	P	T	T	T	T	..	..	52 d
87	224	..	..	..	..	T	T	..	..	107 d
104	236	P	P	T	T	T	T	..	..	52 d
114	350	P	P	T	T	T	T	..	..	69 d
115	370	P	P	P	P	T	T	..	..	71 k
116	305	P	P	T	T	T	T	S	..	76 d
117	330	P	P	T	T	T	T	..	..	68 k
119	200	P	P	T	T	T	T	..	..	57 d

P = partial; S = slight; T = total.

Rats nos. 64 to 105 were given one injection of 0.8 mg., and rats nos. 87 to 119 0.6 mg. of mercuric chloride, with 4 cc. of sodium ferrocyanide simultaneously and 4 cc. daily thereafter for 3 days or until the rat died.

No.	Weight in gm.	1	2	3	4	5	6	7	8	Died, killed, hours
107	307	P	T	T	T	T	T	P	..	54 d
108	339	P	T	T	T	T	T	P	..	58 d
109	325	P	T	T	T	T	T	..	..	49 d
106	334	..	P	T	T	T	T	..	..	62 k
110	359	S	S	P	P	P	P	P	..	56 k
112	348	P	P	T	T	T	T	S	S	60 d

P = partial; S = slight; T = total.

Rats nos. 107 to 112 were given one injection of 0.6 mg. of mercuric chloride with 4 cc. of a mixture of equal parts of sodium ferrocyanide and ferric ammonium citrate (nos. 107 to 109); a second injection of 4 cc. of the mixture 24 hours later (nos. 106 and 110) and a third, like injection, 48 hours later (no. 112).

No.	Weight in gm.	1	2	3	4	5	6	7	8	Died, killed, hours
30	256	..	..	..	..	T	T	..	..	75 d
100	370	S	S	T	T	T	T	..	..	56 d
101	320	S	S	P	P	P	T	..	..	75 k

P = partial; S = slight; T = total.

Rats nos. 30 to 101 were given one injection of 0.8 mg. of mercuric chloride with 2 cc. of ferric ammonium citrate simultaneously and 2 cc. daily thereafter for 2 days.

No.	Weight in gm.	1	2	3	4	5	6	7	8	Died, killed, hours
68	260	P	T	T	T	T	T	..	..	114 d
69	155	P	T	T	T	T	T	..	..	83 d
77	250	P	T	T	T	T	T	..	..	104 d
84	230	P	T	T	T	T	T	T	..	70 d
85	226	P	T	T	T	T	T	..	..	77 d

P = partial; T = total.

Rats nos. 68 to 85 were given one injection of 0.8 mg. of mercuric chloride with 3 cc. of sodium thiosulfate simultaneously; 3 cc. 24 hours later (nos. 77 to 85) and a like amount 48 hours later (nos. 84 and 85).

the use of mercurial diuretics was the following: in two rabbits (nos. 2 and 4), in addition to necrosis of the cells of the portion indicated above, which 3 months after the initial injection of $HgCl_2$ appeared as though recently necrotized, the glomeruli were markedly damaged, their capsular spaces were enlarged and the neck of the tubule was occlusively constricted (Fig 4A). Such occlusion was not observed in the kidneys of the other rabbits studied or in those of the guinea pig and rat. It is possible that a like occlusion would have occurred in the kidneys of most of these animals if they had lived long enough after the $HgCl_2$ was injected.

2. Rats and Frogs. Acute segmental damage involved most frequently the second and third quarters while adjacent segments were less frequently involved (Figs. 2A and 6; 2B, 7 and 8—rats; 9, 10 and 11—frogs). In the frog, segmental damage varied considerably but the initial and terminal portions were less frequently damaged, the neck segment being the most resistant part of the tubule.

Remarks

1. Of unknown significance is the fact as shown here tabularly that only in the kidney of the guinea pig and frog does necrosis of the terminal portion of the proximal convolution occur frequently (guinea pig, 63 per cent; frog, 54.5 per cent) while necrosis of this portion in the kidney of the human, rabbit and rat has not been observed. Necrosis of the entire proximal convolution is rare despite the amount of $HgCl_2$ given or the period of the animal's survival thereafter. Of the animals studied, it has been observed in the kidney of only one guinea pig and in that of four frogs.

Frequency of Total Necrosis of Segments of Proximal Convolution, Tabulated by Eighths

		1	2	3	4	5	6	7	8
Rabbits	(6)	1	1	1	2	2	6	6	0
Guinea pigs	(8)	1	1	1	1	3	5	5	5
Rats	(43)	3	13	29	30	39	40	8	0
Frogs	(24)	9	13	16	16	18	18	15	13
Total	(81)	14	28	47	49	62	62	34	18

Figures in parentheses indicate the number of animals used.

The Degree of Segmental Damage of the Proximal Convolution, Tabulated by Eighths for All the Animals Studied

	1	2	3	4	5	6	7	8
None	33	28	18	16	9	7	36	56
Slight	10	10	6	6	7	5	7	6
Partial	25	16	11	11	3	2	6	3
Total	15	29	48	50	64	69	34	18

2. It has been noted by Crabtree [4] in the kidney of the mouse, and by others cited by her, that in several species of mammals the cells of the parietal layer of some glomerular capsules are normally cuboidal or columnar and that the number of such capsules increases in response

No.	Weight in gm.	1	2	3	4	5	6	7	8	Died, killed, hours
36	55	..	..	T	T	T	T	T	T	106 k
43	50	P	T	T	T	T	T	T	T	98 d
51	50	P	P	T	T	T	T	T	T	156 d
65	55	S	S	S	S	S	S	T	T	200 d

P = partial; S = slight; T = total.

Frogs nos. 36 to 65, in order, were given milligrams of mercuric chloride as follows: 0.56, 1.0, 0.6 and (no. 65 only) 0.6 on the 7th day after a like, 1st injection. Each frog was given, with the mercuric chloride, 3 cc. of sodium ferrocyanide simultaneously and 3 cc. of it daily thereafter for 4 days (nos. 36 and 43) and for 5 and 7 days, respectively (nos. 51 and 65).

No.	Weight in gm.	1	2	3	4	5	6	7	8	Died, killed, hours
66	54	..	..	..	..	T	T	T	T	100 d
74	25	T	T	T	T	T	T	T	S	100 d
63	50	..	..	..	..	P	P	S	..	320 d
64	55	S	S	P	P	T	T	S	..	103 d

P = partial; S = slight; T = total.

Frog no. 66 was given 0.6 mg. of mercuric chloride with 4 cc. of sodium thiosulfate simultaneously and 4 cc. of it daily thereafter for 4 days. No. 74 was given 0.4 mg. of mercuric chloride with 4 cc. of a mixture of equal parts of thiosulfate and urea simultaneously and 4 cc. of it daily thereafter for 3 days. No. 63 was given on the 1st, 6th and 12th days, 0.6 mg. of mercuric chloride and no. 64, one injection of 0.6 mg. of mercuric chloride with 4 cc. of a mixture of equal parts of sodium ferrocyanide and sodium thiosulfate, simultaneously, and 4 cc. of it daily thereafter for 10 days (no. 63) and for 5 days (no. 64).

No.	Weight in gm.	1	2	3	4	5	6	7	8	Died, killed, hours
15	50	..	..	S	S	T	T	T	T	49 k
40	48	T	T	T	T	..	..	..	..	107 k
58	45	..	..	..	..	..	..	P	P	153 k

P = partial; S = slight; T = total.

Frogs nos. 15 to 58, in order, were given milligrams of mercuric chloride as follows: 0.4, 0.56 and 0.4 with 3 cc. of ferric ammonium citrate simultaneously and with 3 cc. of it on the 2nd day (no. 15) and 3 cc. of it daily thereafter for 4 days (no. 40) and 6 days (no. 58).

Notes

These concern the proximal convolution since other portions of the tubule were not significantly damaged.

1. Rabbits and Guinea Pigs. The chief site of acute injury was a quarter between the fifth and terminal eighths (Figs. 1 and 4B—rabbits; Fig. 5—guinea pigs). Segmental damage in the kidney of the guinea pig was variable, as is shown in the tabulated data.

A unique finding and one of possible significance in connection with

to be poorly correlated (a) with the amount of $HgCl_2$ injected; (b) with the size of the animal; or (c) whether the $HgCl_2$ is injected alone or with certain solutions.

4. The frog may be given ten to twenty times more $HgCl_2$ per body weight than may, with similar results, be given the mammal. The frog readily becomes edematous after the injection of effective amounts of $HgCl_2$. This does not occur in the mammal regardless of the amount of $HgCl_2$ injected.

5. The length of the lesion as measured in the third quarter of the proximal convolution varies with the length of the convolution in a given kidney.

Summary

1. Fifty-nine mammals and 24 frogs were injected with mercuric chloride. Some of these were injected with mercury alone or with mercury and certain other solutions in an effort to determine whether the site, extent, or degree of renal injury could thus be modified. The results of such injections are, in general, equivocal and those results indicative of some consequent and specific modification have not seemed to justify the large amount of tedious work that would be necessary in order to establish whether or not modification occurred.

2. The proximal convolution is the only subdivision of the renal tubule in which significant damage is observable after the injection into the various animals studied of usually lethal amounts of $HgCl_2$. Within this convolution a major aspect of such damage is the acute or total segmental necrosis of its cells. This necrosis is indicated below as percentages of involved eighths of the proximal convolution in the kidney of mammals and frogs.

	Eighths							
	1	2	3	4	5	6	7	8
Mammals	10	27	55	58	78	90	36	8.5 per cent
Frogs	38	55	67	67	75	75	63	55 per cent
	10	30	61	65	85	94.5	32	0 per cent

The figures of the last line give the average percentage for the human, rabbit and rat's kidney since, in contrast, the terminal portion of the proximal convolution is totally necrotic in the kidney of 65 per cent of the guinea pigs and in that of 55 per cent of the frogs.

3. Subsequent to the injection of $HgCl_2$, hypertrophy and/or hyperplasia of one-half or more of the outer layer of the glomerular capsule is common in the kidneys of all the animals studied except the rabbit where it is slight and infrequent.

4. Obvious injury to the cells of a part or of all of the distal convolution is rarely seen and there is functional evidence that such aspects of injury as may be observed are more apparent than real. The cells of the thin segment of the loop of Henle are too squamous to permit statements concerning their possible injury and those of the ascending limb are not appreciably damaged.

to certain factors.[5-8] In connection with the renal action of mercury a marked increase was noted in the number of glomerular capsules whose parietal layer was composed of cuboidal cells for varying distances from their junction with the proximal convolution to the vascular pole of the glomerulus (Figs. 3 and 7). These cells constituted in sections of renal corpuscles, structures resembling some of those designated as "epithelial crescents." The capacity for hypertrophy and hyperplasia seemed especially inherent in the cells of the parietal rather than in those of the visceral layer of the capsule. Marked damage of the cells of the initial portion of the proximal convolution was not accompanied by hypertrophy of those of the capsule's parietal layer. Hypertrophy or hyperplasia of the cells of the latter in the kidney of mercurialized animals may be correlated rather with segmental tubular damage than with obvious glomerular damage. A similar hypertrophy in animals treated with sex hormones may be contingent upon metabolic factors.

3. Mitotic figures have not been observed in the hyperplastic, parietal layer of the glomerular capsule nor has epithelial regeneration been observed in connection with the tubular lesions induced by $HgCl_2$. However, pertinent studies with regard to such regeneration and associated phenomena in uranium nephritis and in Bright's disease are those of Oliver and his co-workers.[9-12]

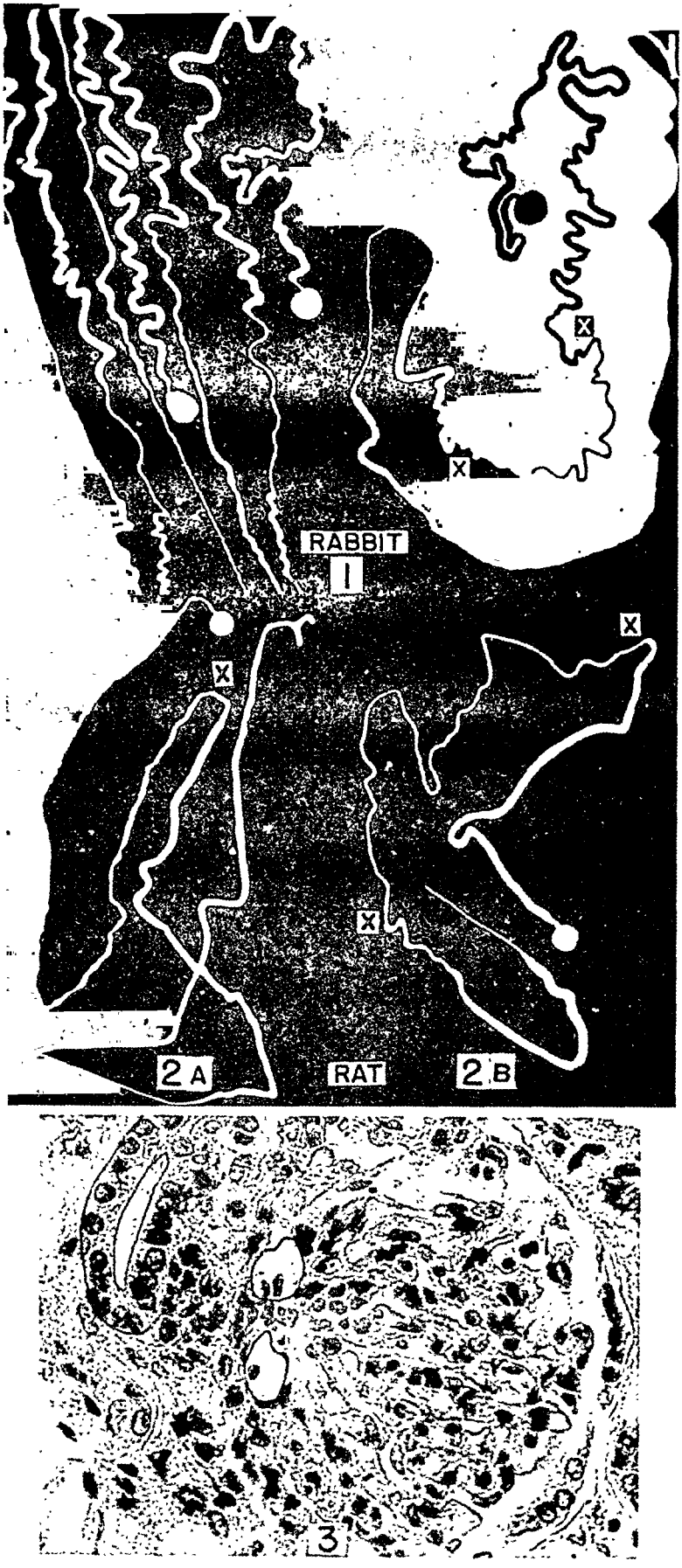
Generalizations

With regard to the animals studied and to the renal tubular damage resulting from the action of mercury, the data of this paper permit the following generalizations:

1. The initial and terminal portions of proximal convolutions are least and less frequently injured than are the intervening portions of such convolutions. The terminal segment (that segment terminating abruptly at its junction with the squamous-celled thin segment of the loop of Henle in mammals or with the cuboidal-celled, intermediate segment in frogs) is less frequently injured than is the initial segment except in the kidneys of the guinea pig and frog.

2. Segmental susceptibility of the proximal convolution to injury is least to greatest with abrupt transitions, from the end of the first eighth to the beginning of the terminal fourth or less. Necrosis of the cells of the most highly susceptible third quarter is often not accompanied by obvious injury to the remainder of this convolution. Also, in the cells of this quarter in the mammalian kidney, iron is normally demonstrable whether or not it has been previously injected into the animal.

3. The extent or degree of the necrotizing action of mercury appears



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DESCRIPTION OF PLATES

PLATE 157

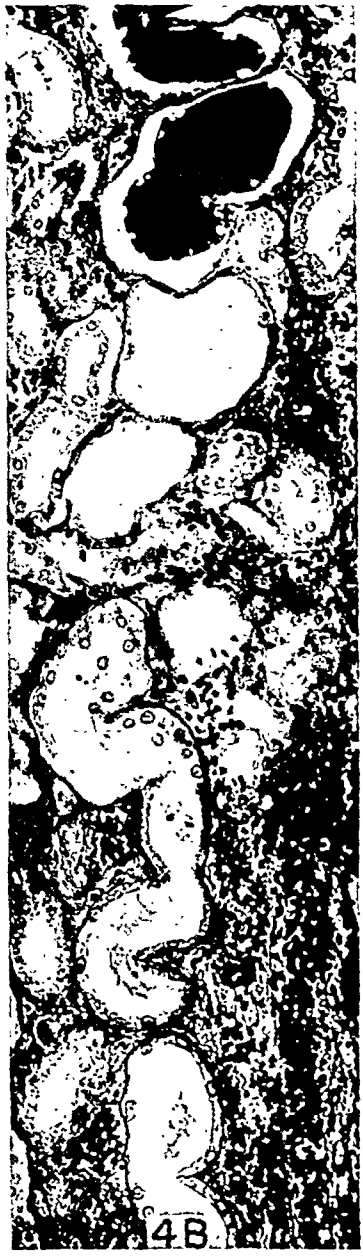
(For all animals referred to by number in the figures, see tabulated data of the text.)

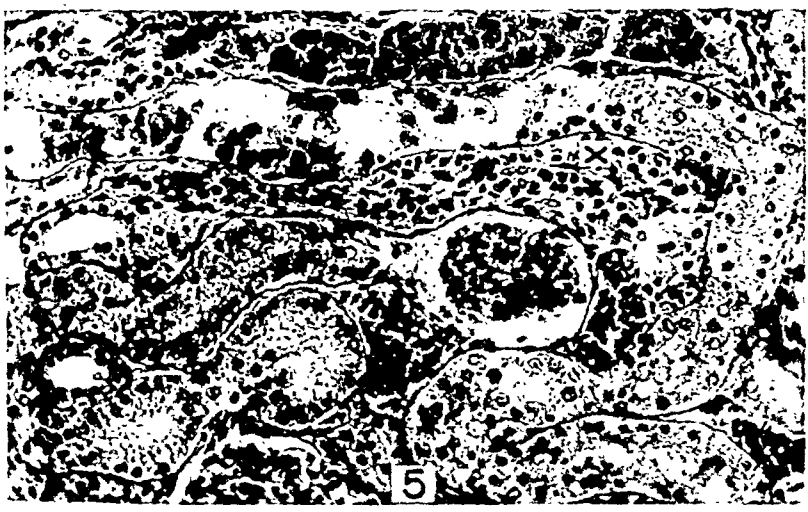
FIG. 1. Photomicrograph showing, left to right, the terminal portions of three and all of two proximal convolutions and one complete renal unit (glomerulus and tubule). These were isolated from the kidney of a rabbit which 4 months previously had been given, by injection, 16 mg. of $HgCl_2$ (rabbit no. 4). The site and extent of injury to the tubule at the right (see, also, Fig. 4B) are shown from X to X, and to the convolutions at the left, by their thin portions. The terminal filament of the third segment from the left is the normal, thin segment of the loop of Henle. × 50.

FIGS. 2A and 2B. In Figure 2A is shown a complete renal unit, and in 2B, a proximal convolution, which were isolated from the kidneys of rats nos. 79 and 95, respectively. The site and extent of total necrosis are shown in 2A, from the glomerulus to X, and in 2B, from X to X. × 50.

FIG. 3. Section of a human renal corpuscle in the kidney of a person dying of mercuric chloride poisoning. At the right is shown a crescentic structure resulting from hyperplasia of the cells of a portion of the outer layer of the glomerular capsule. At the left is shown an oblique section of the initial portion of a normal distal convolution. × 500.





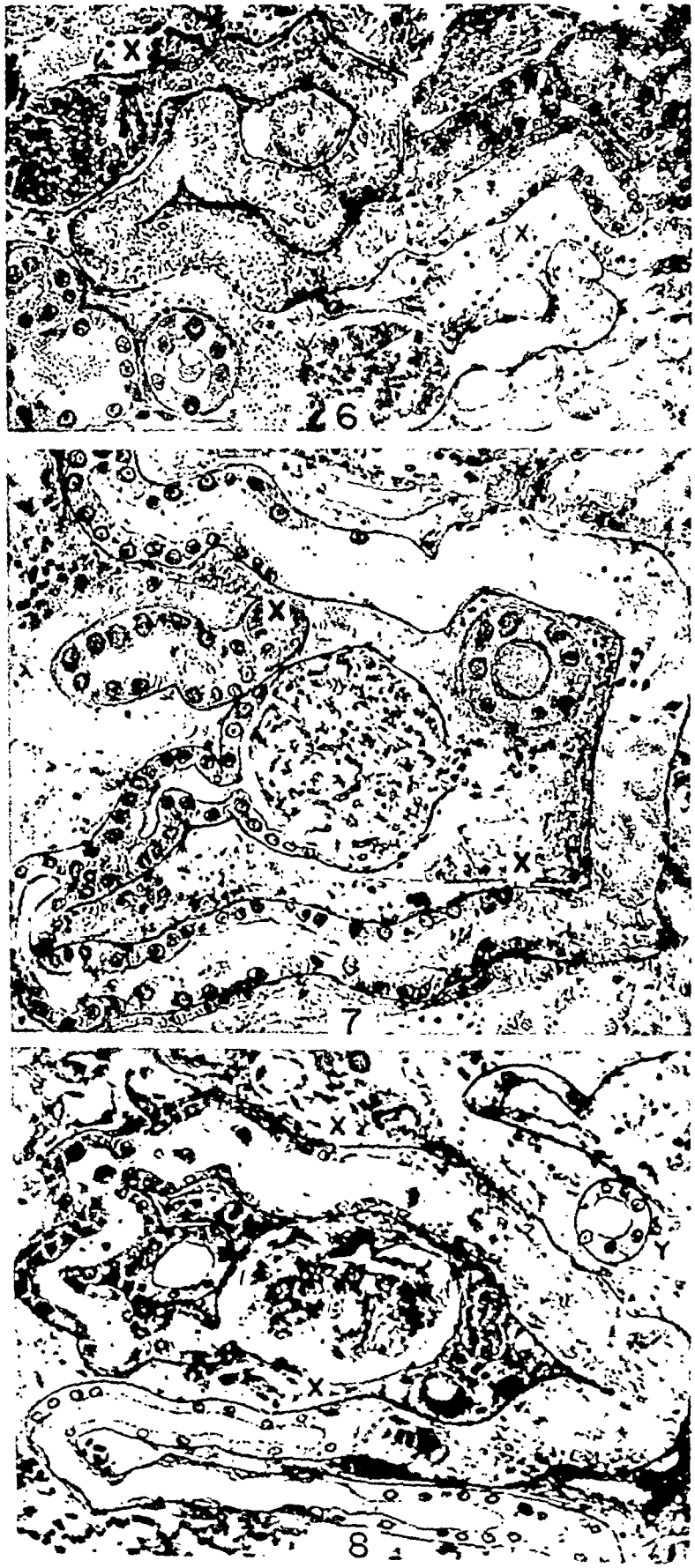


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PLATE 158

FIGS. 4A and 4B. Sections of the cortex of the same rabbit's kidney as that from which Figure 1 was obtained. In A, the upper portions of proximal convolutions and two renal corpuscles are to be noted. The cells of these portions are not obviously damaged but the upper glomerulus is shrunken (the predominant condition of the glomeruli in this kidney and in that of rabbit no. 2). The outlet to the tubule from both glomeruli is constrictively occluded by connective tissue. In B, is shown the character of the lesion involving a part of the third quarter of the proximal convolution. × 300.

FIG. 5. A photomicrograph of composite sections of a guinea pig's kidney (guinea pig no. 7). The sections were so arranged as to show in a small area such features as otherwise would require several photomicrographs. In the center is a glomerulus and at its left, and continuous with its capsule, is the initial and here somewhat U-shaped segment of the undamaged first quarter of the proximal convolution. Below the glomerulus and at the right of the numeral is shown a looped portion of the partly necrotic second quarter. In the upper right bend of the latter is seen the rather abrupt transition between it and the totally necrotic terminal half. Portions of this and of another terminal half, similarly necrotic, extend across the top of the figure. × 200.



Edwards The Renal Tubule as Affected by Mercury

PLATE 159

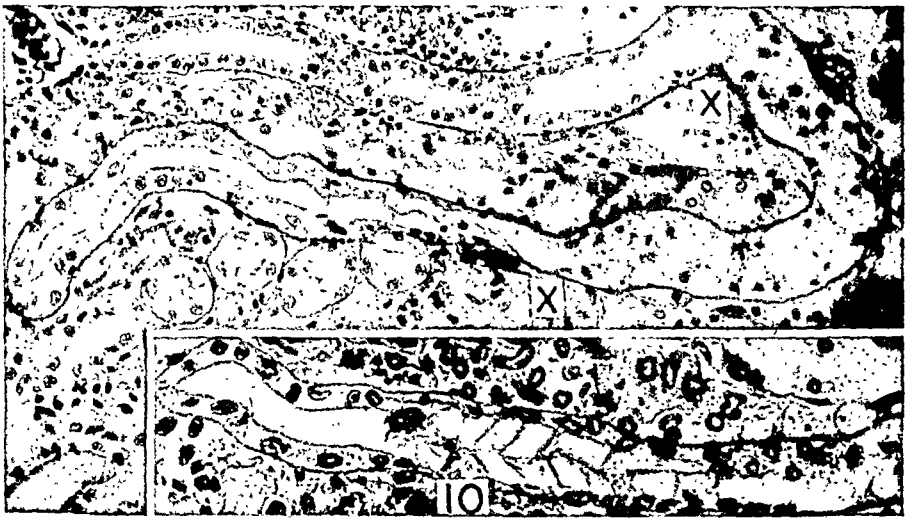
The figures of this plate are of sections of the kidneys of mercurialized rats. They were produced by combining several photomicrographs of different parts of the same proximal convolution within a given kidney so as to represent in miniature the chief site and extent of injury to this convolution in general throughout the kidney.

FIG. 6. Rat no. 79. Here is shown from X to X and also at the right of the lower glomerulus, the necrotic portion which is typical of the first five-eighths of the convolution and above the X at the right, the transition from the necrotic to the relatively nonnecrotic portion. × 150. At the lower left are shown three transections, the central one of which is of a distal convolution and the other two are of its ascending limb. × 300.

FIG. 7. Rat no. 95. This shows (a) at the left of the glomerulus the hypertrophic cells of the outer layer of the glomerular capsule; (b) the totally necrotic segment which, from X to X and abruptly continuous at both of its ends with partly necrotic segments, is typical of the second and third quarters; (c) a transection of a distal convolution at the right of the glomerulus. × 300.

FIG. 8. Rat no. 64 or 104. At the left of the glomerulus and beginning with its capsule, there are portions of a proximal convolution as follows: (a) partly necrotic and typical of the first quarter; (b) totally necrotic as shown at the right from the X above to the X below the glomerulus and typical of the second and third quarters; (c) the relatively undamaged terminal portion which, below the glomerulus, composes the upper left half of the loop and is continuous at its crest with that part of the thin segment which composes the lower left half. The latter is succeeded at the right by the initial part of the ascending limb. A transection of this limb is shown at the left of Y in the upper right portion of the figure. × 200.


X
X
9


X
X
10

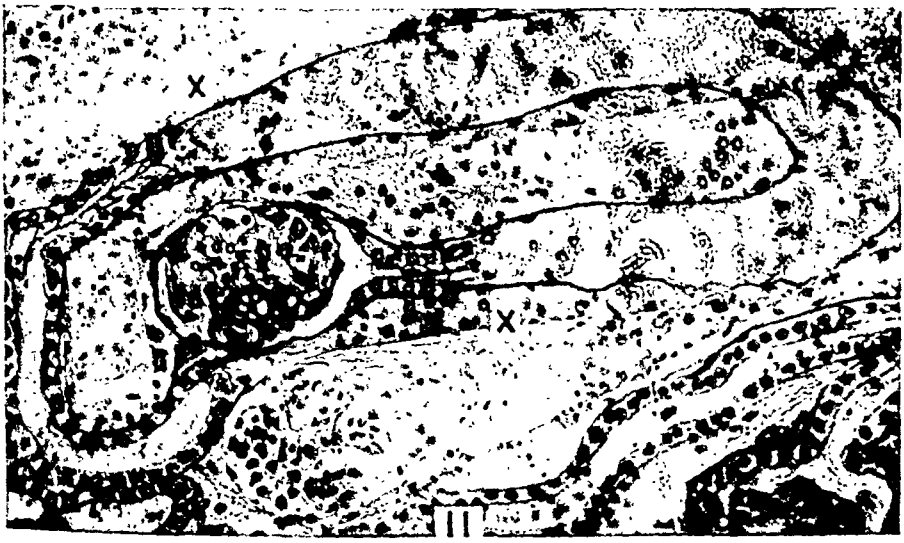

X
X
11

PLATE 160

The figures of this plate were made as in Plate 159 by combining parts of photomicrographs of several sections of particular kidneys from mercurialized frogs so as to represent in each figure the typical aspects of tubules segmentally damaged by the action of mercury.

FIG. 9. Frog no. 64. Variable damage of the proximal convolution is indicated by typical segments of approximately equal lengths extending from the glomerulus at the upper right to the termination of the convolution beneath the upper X. The cells of the initial and distal portions are least injured as shown, respectively, from the glomerulus to the tubular constriction at the upper left and by the portion between and immediately to the left of X and X. Each of these segments constitutes about one-sixteenth of the entire convolution. From the constriction mentioned to the first X below, a degree of damage is shown which is typical of the first and second quarters except the initial sixteenth of the first quarter. The greatest injury, involving the cells of the third and most of the fourth quarter, is shown, respectively, by the upper and lower halves of the segment between X and X. Parts of two intermediate segments, *i.e.*, segments between the ends of proximal and the beginning of distal convolutions, are shown: one is beneath the upper X and the other extends from the numeral upward to the first slight curve where it becomes abruptly continuous with the initial portion of a distal convolution. The latter, and like convolutions throughout this and the kidney of other mercurialized frogs, are not significantly damaged despite the inadequacy of the figure as proof. × 300 (lower left quarter, × 200).

FIG. 10. Frog no. 69 or 75. There is shown from the upper left to the X at the upper right, the undamaged portion which is typical of the first eighth of the proximal convolution, and between X and X, the necrotic portion which is typical of the remainder of this convolution. Also, above the lower X and above and to the right of the numeral, there are parts of intermediate segments which are continuous at the left with initial portions of normal distal convolutions. These convolutions are, in general, normal in kidneys which have been damaged by mercury. × 200 and (inset) 350.

FIG. 11. Frog no. 82 or 84. From X to X in the upper right half, the total necrosis is typical of the entire proximal convolution except the normal neck segment. The latter is shown between the glomerulus and lower X. At the left of the upper X is an intermediate segment which is abruptly continuous at the extreme left with the initial portion of an undamaged distal convolution. In the lower right corner the sharply bent, terminal portion of a distal convolution joins a collecting duct. × 300.

THE ORIGIN OF COLLOID AND LIPOID DROPLETS IN THE EPITHELIAL CELLS OF THE RENAL TUBULES *

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The origin of colloid droplets in the lining cells of renal tubules has long been the subject of controversy. According to Aschoff,[1] colloid droplets are an expression of an inflammatory reaction of the cytoplasm (tubular nephritis). According to Fahr,[2] colloid droplets represent the secondary effect of a primary injury to the renal epithelium. Laas [3] distinguished between infiltrative and degenerative colloid droplets depending on whether they are due to storage of protein or disintegration of cytoplasmic protein. Terbrüggen [4] interpreted colloid droplets as an expression of disturbed secretion of tubular cells.

On the basis of results obtained in experiments with urodeles (*Salamandra maculosa*) Gérard and Cordier [5-8] and Lambert [9-11] concluded that colloid droplets represent protein material which has been taken up from the lumina of the tubules and stored by the tubular cells (athrocytosis). By analogy they interpreted the presence of colloid droplets in human renal disease as secondary to a functional change of the glomeruli permitting the passage of protein into the tubules.

Hein [12] and Kleier,[13] repeating and expanding the studies of Gérard and Cordier,[5-8] were able to confirm their findings. A comprehensive survey of the various theories and opinions concerning the formation, character and significance of colloid droplets in the renal tubules was given by Randerath.[14]

The problem was taken up by us in order to reinvestigate several points in the production of colloid droplets by employing a technic which made it possible to label the materials to be used. This seemed desirable because preliminary studies of the kidneys of five normal salamanders and three normal necturi had revealed unmistakable colloid droplets in the "open" and "closed" nephrons. These "natural" colloid droplets were quite distinct from the lipochrome granules and droplets occurring in the central portion of tubular cells of the "open" and "closed" nephrons.[6]

MATERIALS AND METHODS

We were unable to procure specimens of *Salamandra maculosa* and therefore employed *Salamandra punctata* (*Amblystoma punctatum*) and *Necturus maculatus* for our experiments. Both of these urodeles

* Received for publication, February 17, 1942.
† Deceased.

For certain experiments human sera, unlabelled by coupling with dyes, from cases of lipoid nephrosis, containing 950 mg. per cent and 860 mg. per cent respectively of cholesterol and horse serum (total protein, 12.8 mg. N per cc.; albumin, 5.2 mg. N per cc.; globulin, 7.4 mg. N per cc.) were used.

Groups of animals were injected intraperitoneally 1 to 4 times with 0.5 cc. of the various preparations and the animals were then sacrificed after different periods of time ranging from 2 to 328 days. The tissues were fixed in Zenker's fluid containing 5 per cent of glacial acetic acid, and in a 4 per cent solution of formaldehyde. Paraffin sections of animals having received R salt preparations were stained with hematoxylin only, while those with multiple dye injections were examined unstained. Portions of formaldehyde-fixed kidneys were cleared according to the method of Spalteholz [20] so that the entire length of the tubules of the "open" nephrons could be studied.

a. The Distribution of Protein-R Salt Compounds in the Organs of Urodeles After Intraperitoneal Injection

All protein-R salt preparations mentioned above, including peptone, placed into the peritoneal cavity of 6 *Amblystoma punctatum* and 24 *Necturus maculatus,* produced intensely red granules or droplets in the lining cells of the tubules of the "open" nephrons only. In no instance were colored particles seen in the cells of the "closed" nephrons (Figs. 1 and 2). In addition, colored granules were usually present in the Kupffer cells and occasionally in some of the reticuloendothelial cells of the spleen. Sometimes phagocytic cells, holding colored granules, were seen in the peritoneal layer of abdominal organs. Necturi, injected with necturus serum albumin or globulin-R salt preparations, showed colloid droplets in the tubular cells of the "open" nephrons but did not show colored granules in the Kupffer cells. Preparations of segments of necturus kidneys cleared according to the method of Spalteholz [20] never revealed colored material in any of the "closed" nephrons while the "open" tubules were always clearly defined by their intense red color.

Similar results were obtained after injections of proteins coupled with m- or p-malachite green.

Colored granules and droplets were also seen in the "open" nephrons after the intraperitoneal injection of diazotized R salt alone. This result was quite puzzling until it was found that the fluid normally present within the peritoneal cavity of the necturus contained 0.65 per cent protein. The R salt injected had combined with this protein and then produced colored colloid droplets. The presence of protein-containing peritoneal fluid easily explained the occurrence of "natural" colloid droplets seen in untreated animals.

are equipped with nephrostomial canals connecting the peritoneal cavity with the "open" nephrons, so that the experimental conditions are comparable to those in *Salamandra maculosa*. The kidney of necturus is especially well suited for this work because of the single segmental "open" nephrons which are easily identified.

The various proteins to be used were coupled with the disodium salt of 2-naphtol-3:6 disulfonic acid (R salt)* according to the procedure described by Kabat and Heidelberger.[15] After having twice precipitated the dye-protein compounds with acetic acid, the precipitate was dissolved in saline of pH 7 and then dialyzed against saline of the same pH until no more uncombined dye passed through the membrane. One per cent by volume of a 1 per cent solution of merthiolate was added to each preparation as a preservative. Nitrogen determinations were made with the micro-Kjeldahl method in duplicate.

The protein-R salt compounds are quite stable and can easily be identified, grossly as well as microscopically, by their intense red color.

The preparations used in the experiments were as follows:

Neopeptone (Difco Laboratories, Detroit, Michigan)—R salt, 0.2 mg. N per cc.
Egg albumin (thrice recrystallized)—R salt, 6.8 mg. N per cc.
Serum albumin (dog)—R salt, 0.3 mg. N per cc.
Serum albumin (necturus)—R salt, 0.2 mg. N per cc.
Serum globulin (dog)—R salt, 0.3 mg. N per cc.
Serum globulin (necturus)—R salt, 0.4 mg. N per cc.
Serum globulin (cat)—R salt, 1.8 mg. N per cc.
Casein—R salt, 1.1 mg. N per cc.
Diazotized R salt alone, 0.12 per cent.

In addition to R salt, preparations of dyes of the malachite green series[16-18] were coupled with various proteins. In this manner protein-dye compounds of green color (m-malachite green) and of blue color (p-malachite green) were obtained. The dyes were coupled to the proteins according to the method of Heidelberger:[19] 0.34 gm. of m- or p-NH_2 leukomalachite green was dissolved in 25 cc. of H_2O to which were added 2 cc. of 1:1 concentrated HCl. After chilling, the dye was diazolized with 0.08 gm. of $NaNO_2$, dissolved in 5 cc. of H_2O. 17 cc. of a suspension of $PbCO_3$ (containing 14.6 mg. per cc.) were added with constant stirring. After a few minutes, 0.2 gm. of Na_2SO_4 were added, after which the solution was centrifugated to remove the lead sulfate.

290 cc. of a solution of NH_3 free thrice recrystallized egg albumin containing 10.4 mg. protein per cc. were diluted to 500 cc. and chilled. 25 cc. of 2N Na_2CO_3 were added and 70 cc. of the above-mentioned diazo solution were allowed to run in drop-wise with constant stirring. A similar technic was used in the preparation of other protein-dye compounds.

The following protein-dye compounds were prepared by this procedure:

Neopeptone (Difco Laboratories, Detroit, Michigan)—p-malachite green, 0.86 mg. N per cc.
Crystallized egg albumin—m-malachite green, 0.7 mg. N per cc.
Serum globulin (sheep)—m-malachite green, 0.82 mg. N per cc.
Casein—p-malachite green, 1.3 mg. N per cc.

*The R salt was supplied through the kindness of Dr. L. M. Crossley of the Calco Chemical Company.

"open" nephrons stood out clearly, due to their red color (Fig. 4). The coloration, which was due to the presence of red granules or droplets within the lining cells, began at the point where the nephrostomial canal joins the lumen of the proximal convoluted tubule, and ended at the junction between the distal convoluted tubule and the collecting tubule (wolffian duct). Sometimes a faint coloration extended into the neck of the proximal portion of the convoluted tubule and rarely was color seen in the space of Bowman of the glomeruli of the "open" nephrons. In no instance was there any specific location of storage within the "open" nephrons. The intensity of the color varied according to the time elapsing since the last injection and seemed proportional to the amount of material taken up by the lining cells. There was, however, no difference in the intensity of color in different portions of the "open" nephrons. As stated above, the "closed" nephrons were always free of colored particles.

(2) *Multiple Protein-Dye Injections.* Four series of experiments were performed with groups of three necturi each. In two series, mixtures of three proteins coupled with different dyes were injected intraperitoneally, while in the other two series, three protein-dye compounds were injected separately, spacing the injections 1 week apart.

The combinations of materials used in these experiments were as follows:

Series 1. Peptone-p-malachite green, egg albumin-R salt, sheep serum globulin-m-malachite green. One cc. of a mixture of these three compounds was injected on 3 consecutive days.

Series 2. One cc. of each of the three compounds used in series 1 was injected separately with an interval of 1 week between injections.

Series 3. One cc. of a mixture of the following dye-protein compounds was injected on 3 consecutive days: egg albumin-m-malachite green, cat serum globulin-R salt, casein-p-malachite green.

Series 4. One cc. of each of the compounds used in series 3 was injected separately, spacing the injections 1 week apart.

In addition, a few necturi were injected with similar dye-protein combinations, spacing the individual injections approximately 1 month apart. The animals were sacrified after periods of time varying from 2 days to 36 days after the last injection.

Histologic preparations of the kidneys of necturi of series 1 and 3 showed uniformly colored granules and droplets in the cells of the tubules of the "open" nephrons. The color was the resultant of the mixture of the three colors—red, green and blue. Kupffer cells contained small granules of the same color. No colored material was seen in any of the tubules of the "closed" nephrons. Cleared segments of

b. The Relationship Between the Character of Colloid Droplets in the Renal Epithelium and the Substances Employed to Produce Them

The size of colloid droplets varied according to the substances employed as well as to the time elapsing between the intraperitoneal injection and observation. Newly formed colloid droplets were always large, rather faintly tinted and occupied the supranuclear zone of the cytoplasm of the tubular cells. After some time they became smaller, darker in color and were situated in the middle and basal portions of the cytoplasm where they attained a certain relative uniformity. The constantly largest colloid droplets were seen in experiments with globulins (Fig. 3) and with a 20 per cent suspension of casein-R salt in saline, while solutions of casein-R salt produced rather small granules and droplets. The most uniform granules of small size were observed with egg albumin-R salt preparations. Serum albumin produced both large and small droplets as did peptone. There was no constant relationship between the size of the colloid droplets and the molecular weight of the substances employed, which varied from about 20,000 (peptone) to over 100,000 (globulin, casein). We were unable to confirm the statements of Lambert [11] and Hein [12] that intraperitoneal injection of split products of proteins causes formation of tiny droplets in the proximal portion of the convoluted tubules of the "open" and "closed" nephrons. In our experiments with peptone-R salt preparations no colored droplets were seen in the cells of the "closed" nephrons, although the size of the peptone molecule would permit its passage through the glomerular filter.

c. The Site of Storage of Colored Particles within the "Open" Nephrons in Relation to the Molecular Weight of the Substances Employed

According to Gérard and Cordier,[8] Lambert [11] and Hein [12] the site of storage of proteins or dyes within the nephrons depends on the molecular size or dispersion of the substances employed: the larger the molecule, the more distally it is stored.

The validity of this statement was tested in several ways:

(1) *The Site of Storage of Protein-R Salt Compounds of Different Molecular Weights.* Twenty-seven necturi were injected intraperitoneally 1 to 4 times with 0.5 cc. of different protein-R salt preparations varying in molecular weight from about 20,000 to over 100,000, and were then sacrificed after a varying length of time. Segments of kidneys of such treated animals were cleared according to the method of Spalteholz [20] so that the length of the tubules of "open" and "closed" nephrons could be seen. In all of these preparations the tubules of the

droplets within the cells, first from the supranuclear zone to the infranuclear zone and then back towards the lumen just before their disappearance; he also pointed out a vacuolar change of the cytoplasm after the disappearance of colloid droplets. According to Lambert,[9] colloidal dyes taken up by renal epithelial cells disappear very slowly, if at all, and this is contrasted by Hein with the rapid disappearance of colloid droplets.

The reinvestigation of the fate of the colloid droplets was complicated by two factors: the occurrence of spontaneous colloid droplets, on the one hand, and on the other, the possible interference of the coupled dye with the digestion of colloid droplets. For this reason we studied the fate of colloid droplets produced by labelled as well as unlabelled materials, and compared the results.

Ten necturi were injected intraperitoneally with 0.5 cc. of horse serum on 4 consecutive days and the animals were sacrificed at intervals 7 to 67 days after the last injection. Small series of sections of the kidneys were stained with hematoxylin and eosin and were thoroughly examined for the presence of colloid droplets in the "open" nephrons.

Unmistakable colloid droplets of various sizes were present in the tubular cells of the "open" nephrons in all. Their number varied but this did not seem to be definitely related to the time elapsed. The largest number of colloid droplets in this series of experiments was seen in animals sacrificed 32 and 39 days after the last injection of serum. No signs of digestion of colloid droplets could be made out; there were, however, a few vacuoles within the cytoplasm of the tubular cells of the "open" nephrons. Frozen sections stained with scharlach R showed these to be fat droplets.

In the experiments conducted with the above-mentioned protein-R salt (sections a and b) colored colloid droplets were present in the tubular cells of the "open" nephrons in all instances from 2 days to 328 days after the last intraperitoneal injection. The character of the droplets changed during this time. In the earliest stage colored protein casts were present in the lumina of the tubules. Some of this amorphous material was then absorbed by tubular cells and segregated into large, faintly tinted colloid droplets just beyond the brush border of the cells in the supranuclear zone of the cytoplasm. Soon afterwards the droplets were moved towards the base of the cells where they became smaller and deeper red in color. Often the supranuclear zone was then free from colored colloid droplets but showed large, untinted, "natural" droplets (Fig. 5). In animals killed within 2 to 3 weeks after the last injection, some of the cells were tremendously distended by large

the kidneys showed the entire length of the tubules of the "open" nephrons uniformly affected.

Histologic preparations of the kidneys of series 2 and 4 showed granules and droplets of different colors in the cells of the tubules of the "open" nephrons only. The droplets having the color of the protein-dye compound injected first were always seen in the basal portion of the cells, those of the last injection towards the lumen and the second one in the central portion of the cells. Individual tubules showed uniform distribution in all parts of the nephron but there were differences in the number of droplets of different color in different nephrons. From the outcome of these experiments, it was concluded that there is no preferred site of storage of protein particles of different character in different parts of the tubules of the "open" nephrons.

In order to prove that in a mixture of three protein-dye compounds the dyes were inseparably attached to their respective proteins, the mixtures used in series 1 and 3 were studied in the electrophoresis apparatus of Tiselius.[21] The mixtures were dialyzed against 0.02 mol. phosphate buffer containing 0.15 mol. NaCl, pH 7.51 and were then run for 2 hours in a standard cell under standard conditions. The electrophoretic mobilities of the boundary lines were studied and the pattern was photographically recorded by means of Longsworth curves[22] and by cylindrical lens curves[23,24] using special lamps and filters to record the different colors of the mixture.

Both preparations showed three boundaries in the ascending and descending limb. Series 1 showed a fast-moving blue component, a slow-moving green component, while the middle portion was of a composite purple color. Series 3 showed a faster moving greenish blue component, a very slow red component, while the middle portion was again of a composite purplish color. The mobilities of the boundaries corresponded to those of the substances contained in the mixtures. The results obtained in these experiments indicated that protein-dye compounds had retained their individual character within the mixture.

d. The Fate of Colloid Droplets

According to Kleier,[13] colloid droplets appear in the cells of the tubules of the "open" nephrons 2 days after the injection of protein material into the peritoneal cavity and disappear in summer animals 10 days, in winter animals 17 days, after the last injection. Concerning the mechanism of their disappearance, Hein [12] speculated whether metamorphosis (*Umbau*) or decomposition (*Abbau*) of the colloid droplets takes place, while Randerath [14] and Kleier [13] spoke of digestion and splitting. Kleier described a change in position of the colloid

of this mixture revealed 1.91 mg. total nitrogen per cc.; 0.33 mg. of nonprotein nitrogen per cc.; 1.07 of noncoagulable nitrogen per cc., and 0.41 mg. amino nitrogen per cc. Twenty-seven cc. of the mixture were placed in a cellophane bag which was surrounded by 40 cc. of a solution of phosphate buffer of pH 8 and kept at 37° C. After 24 hours the total nitrogen content of the fluid within the bag had fallen from 51.4 mg. to 25.8 mg. while that of the outside buffer had risen to 25.6 mg. The nonprotein nitrogen of the digested material was then 2.16 mg. per cc., the noncoagulable nitrogen 2.7 mg. per cc. and the amino nitrogen had risen to 6.9 mg. per cc. A parallel experiment with untreated casein gave comparable results. Several such experiments gave similar results, which indicated that the coupling of the casein to R salt did not interfere with the digestion by trypsin *in vitro*.

The effect of trypsin and pepsin on colloid droplets produced by coupled and native protein was studied by subjecting unfixed frozen sections of kidneys to solutions of the enzymes on the warm stage at 37° C. There was fading out of the color from the colloid droplets after 2 hours following trypsin digestion, while the colloid droplets remained visible for 24 hours. Pepsin neither affected the colloid droplets nor their color.

The Effect of Intravenous Injection of Diazotized R Salt and Protein-R Salt Preparations

One cc. of diazotized R salt was injected into the inferior vena cava of two necturi under anesthesia and the animals were bled to death 6 hours and 16 hours, respectively, after the injection. The serum of the necturus sacrificed after 6 hours showed the deep red color of the R salt; that sacrificed after 16 hours was of light red color. There was no hemolysis in either serum. Sections of the organs of the animals revealed bright red granules in phagocytes in most of the glomeruli and rare, faintly colored granules in the Kupffer cells. No trace of the dye was found in any of the renal tubules of the "open" and "closed" nephrons. The color of the serum showed that the diazotized R salt had combined with the serum proteins but had not passed into the renal tubules.

One cc. of casein-R salt solution was injected under anesthesia into the inferior vena cava of a necturus and the animal was sacrificed 4½ hours afterwards; likewise, 0.3 cc. of the same solution was injected into an *Amblystoma punctatum* and the animal killed 56 hours afterwards. Tiny red granules and faintly colored droplets were found in tubular cells of "open" and "closed" nephrons in both. In another set of experiments two necturi were injected intravenously with 1 cc. of egg albumin-R salt solution and the animals were sacrificed 72 hours

colloid droplets, sometimes causing rupture of the cell membrane (Fig. 6). Occasionally, colored droplets were seen in some of the nuclei of tubular cells. Desquamation of cells distended by colloid droplets was not infrequently observed, especially during the earlier stages, and such discharged cells, with colloid droplets still within their cytoplasm, could be seen within the lumina of tubules. Depending on the amount of coupled material still present in the peritoneal cavity, the regenerated cells showed only a few colored granules or untinted droplets, or none at all.

After about 1 month the colored granules and droplets were all concentrated in the basal portion of the cells and sometimes formed dense segregated lumps. At about the same time colored granules and droplets were observed in cells of the stroma surrounding the tubules of the "open" nephrons (Fig. 7). At first these cells were usually situated just beyond the basement membrane of the tubules and the number and the size of the individual granules, which they contained, varied from a few tiny particles to large colloid droplets or masses of deeply stained red material. The number of these phagocytic cells increased and they seemed more widely scattered through the intertubular stroma the longer the animals were allowed to live. Not infrequently such cells were then seen within lumina of peritubular capillaries and lymphatics, which suggested that they were capable of locomotion.

Since there seemed to be a probable relationship between the labelled colloid droplets within the cells of the tubules and the colored material in the cells outside the tubules, a thorough search was made to find any means of transportation of the tagged proteins through the basement membrane. In several instances close study of sections (stained with a modified Mallory stain to bring out the basement membrane of the tubules) strongly suggested that there exist tiny pores in the basement membranes through which granules can pass and be then taken up by phagocytic cells of the peritubular stroma (Fig. 8).

Although colored granules or droplets were still present in the tubular cells almost 1 year after the last peritoneal injection of labelled materials, their number decreased definitely in the course of time. This was attributed to desquamation of entire cells as well as to a discharge of granules through pores of the basement membranes. There was no evidence of splitting, nor was there ever seen a reverse movement of droplets from the base of the cells towards the lumen of the tubules. The colored granules in the Kupffer cells also remained visible throughout the duration of the experiments.

In order to study the effect of an enzyme on protein coupled with R salt *in vitro*, 10 per cent by volume of a 2 per cent solution of trypsin (Fairchild) was added to a casein-R salt solution of pH 8. An analysis

Six salamanders and 15 necturi were studied in this manner. In addition to paraffin sections stained with hematoxylin and eosin, frozen sections were cut and stained with scharlach R; unstained frozen sections were examined under the polarizing microscope.

In paraffin sections stained with hematoxylin and eosin the cells of the "open" nephrons were large, pale-staining and vacuolated (Fig. 9). Frozen sections stained with scharlach R revealed large numbers of fat droplets of various sizes in the cytoplasm of the cells throughout the entire length of the "open" nephrons, which had the tendency to accumulate in the basal portion of the cells (Fig. 10). Abundant doubly refractive cholesterol crystals and Maltese crosses were demonstrated under the polarizing microscope (Fig. 11). Cells containing fat droplets but no doubly refractive particles were encountered in the stroma surrounding the tubules of the "open" nephrons; their number varied and corresponded generally to the length of time which had elapsed since the last injection. In addition to the fat droplets, the tubular cells of the "open" nephrons showed abundant large colloid droplets. The number of fat droplets in the cells of the "closed" nephrons was not increased and double refraction was never encountered.

The mechanism of the absorption of lipoids from the tubular lumina was similar to that observed after intraperitoneal injection of proteins and formation of colloid droplets. In early cases globular casts of different sizes made up of lipoid-containing serum were seen in the tubular lumina. These were then split up into small droplets beyond the brush border of the lining cells. At first the lipoid droplets filled the entire cytoplasm of the lining cells and later accumulated in the basal portion.

Abundant lipoid droplets, which partly were doubly refractive, as well as colloid droplets, were present in the cells of the "open" nephrons of a necturus 300 days after the last injection of nephrotic serum. Fat droplets, sometimes showing double refraction, were also encountered in the Kupffer cells of the injected animals and in some cases the liver cells seemed to contain more fat than normal.

Discussion

The results of the experiments on urodeles with dye-protein compounds definitely relate the colloid droplets in the "open" nephrons to the various materials injected intraperitoneally. The presence of "natural" colloid droplets was by no means a rare occurrence in our experience, so that the results of previous studies with unlabelled materials cannot be correctly evaluated. The presence of protein in the peritoneal fluid of normal animals adequately explained the occurrence of "nat-

and 1 week afterwards. Abundant colored granules were present in the Kupffer cells 72 hours after the injection and some of the glomeruli contained similar granules in phagocytic cells. The lining cells of the "open" and "closed" nephrons showed small red granules. No evidence of the dye-protein was seen in the kidney 1 week after the injection, while the Kupffer cells showed definite colored granules.

Because of the unexpected passage of casein-R salt through the glomerular filter in urodeles, a study was undertaken to follow the fate of various protein-dye preparations after intravenous injection in mice.

The following solutions were employed: peptone-R salt, egg albumin-R salt, serum albumin (dog)-R salt, serum globulin (cat)-R salt, casein-R salt. Series of five mice each were injected with 0.5 cc. intravenously and the animals sacrificed ½ hour, 1 hour, 2 hours, 3½ hours and 24 hours afterwards.

With the exception of mice having received peptone-R salt, the kidneys of all animals showed tiny, bright red granules in the cells of the proximal convoluted tubules which were more numerous the longer the animals were allowed to live. In the animals killed ½ hour after injection, there were but a few granules in the epithelial cells of the most proximal portion of the tubule at the point of junction with the space itself. The longer the mice were allowed to live after the injection, the farther down the granules extended into the more distal portions of the proximal convoluted tubules. They were most numerous after 24 hours. The Kupffer cells contained similar bright red granules in all experiments including peptone and the granules seemed more numerous in the animals that had lived longer. Although peptone-R salt was excreted into the urine, as evidenced by the dark red color, no colored granules were seen in renal cells. A second series of five mice injected similarly with peptone-R salt showed no granules in the renal epithelium, although the Kupffer cells contained colored material. Studies on the renal excretion and tubular reabsorption of dye-protein compounds in several mammalian species are now under way and will be reported elsewhere.

The Effect of Intraperitoneal Injection of Lipoids on the Renal Epithelium

The lining cells of the "open" and "closed" nephrons of necturus and *Amblystoma punctatum* contain normally a few basally located fat droplets usually situated in the loops of the distal convoluted tubules. Doubly refractive lipids were never encountered in normal animals and therefore preparations, rich in cholesterol, were used to label the materials employed in these experiments. After five intraperitoneal injections of 0.5 cc. each of serum from cases of lipoid nephrosis, the animals were sacrificed from 2 days to 300 days after the last injection.

practically all experiments, these cells were apparently able to retain the materials reaching the liver, perhaps by means of lymph channels, so that they were not disseminated throughout the general circulation. Intravenous injection of coupled egg albumin and casein, however, caused formation of colored particles in the cells of both the "open" and "closed" nephrons, indicating passage of these substances through the glomerular filter.

Laparatomy wounds of necturi and salamanders which were made to inject materials into the vena cava had no tendency to heal. Therefore, mice were used to follow the fate of labelled proteins injected intravenously. Surprisingly, all proteins employed, including dog serum globulin and casein, partly passed through the glomerular filter and were reabsorbed by the lining cells of the proximal convoluted tubules and stored in the form of fine granules and droplets with the exception of peptone, which passed into the bladder urine.

Preparations of serum globulin, injected intraperitoneally, had the tendency to produce rather large colloid droplets in the "open" nephrons. Otherwise there was no constant relationship between the size of the colloid droplets and the substances employed to produce them. In general, the size of the droplets depended more on the time which had elapsed since the peritoneal injection than on the substance employed.

No selective site of storage for any of the proteins within the "open" nephrons could be demonstrated. The entire length of the tubules was equally affected, no matter which preparation was used, and proteins of different molecular weight coupled with dyes of different color were stored in the same cells throughout the "open" nephrons.

The results of experiments conducted to study the phagocytosis and storage of lipids in the cells of the "open" nephrons confirmed those reported by Gérard and Cordier.[7] Fats and lipids never appeared in the "closed" nephrons and the fat droplets and cholesterol remained in the cells of the "open" nephrons as long as did the colloid droplets.

The colloid droplets and fatty changes produced experimentally in urodeles are morphologically indistinguishable from those seen in renal epithelial cells in human cases of nephrosis or nephritis. Although it is perhaps not permissible to apply results obtained in lower animals directly to human beings, the outcome of the experiments nevertheless strongly suggests that colloid droplets are the result of reabsorption and storage of protein from the tubular lumina after passing through the damaged glomerular filtering membrane. The tremendous enlargement, signs of degeneration and desquamation of tubular cells in cases of excessive protein storage indicate that the cells can be damaged during this procedure. Colloid droplets, therefore, are not the result of

ural" colloid droplets in the "open" nephrons, and it may be the normal function of these tubules to reabsorb valuable substances which otherwise would be lost. Colloid droplets were seen 2 days after the intraperitoneal deposition of tagged proteins and their number increased as long as there remained material to draw from. After 1 to 2 weeks the cells usually were filled to capacity so that rupture of the cell membrane sometimes occurred. Desquamation and sloughing of lining cells were also frequently encountered, indicating damage by overloading with colloid droplets. Colloid droplets were formed only within cells from amorphous protein casts within the tubular lumina. The material was absorbed by the lining cells and at first segregated into large globular masses beyond the brush border. Soon these were changed into well rounded colloid droplets which still occupied the supranuclear zone of the cytoplasm. Gradually the droplets became smaller and were moved towards the basal portion of the cells, where they remained. The decrease in size and corresponding increase in color suggested condensation and dehydration of the labelled materials within the cells. The question of the ultimate fate of colloid droplets has to be left open; on the one hand, studies with unlabelled proteins are unsatisfactory because of the occurrence of "natural" colloid droplets, while, on the other hand, the interference of coupling of dyes on splitting and digestion of proteins by cells cannot be ruled out with certainty. The absence of "natural" colloid droplets in the "open" nephrons of many of the animals suggests that colloid droplets can be split and digested, depending perhaps on the amount of protein within the cells. Actual evidence of digestion of colloid droplets within cells was not observed. The vacuoles which Kleier [13] described and which were also seen in our material, occurred only after injections of whole serum and were found to represent fat. Although there was evidence that protein particles and fat droplets could pass the basement membrane of the tubules through small openings, it is rather questionable whether this mode of passage plays an important rôle in the elimination of colloid droplets from the lining cells, since abundant colored colloid droplets were still present in most of the cells 1 year after the last injection. Phagocytosis and storage within cells of the "open" nephrons were not influenced by the kind of protein used. Protein substances of homologous and heterogenous species were taken up in the same manner and remained in the cells for the same length of time.

In no instance was the intraperitoneal injection of coupled protein material followed by the appearance of colored colloid droplets in the lining cells of the "closed" nephrons, even when preparations were used whose molecular size would ordinarily permit passage through the glomerular filter. Although the Kupffer cells showed colored granules in

Protein-dye compounds injected intravenously into urodeles and mice partly pass through the glomeruli and are reabsorbed and stored by the lining cells of the renal tubules.

Parallels are drawn between the morphologic changes seen in human renal disease and those experimentally produced in urodeles.

NOTE: We wish to express our appreciation to Ruth Silbowitz for her technical assistance.

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cell degeneration but they can be the cause of degeneration of tubular epithelial cells.

By analogy, the morphologic changes in the kidneys in human lipoid nephrosis are due to an increased permeability of the glomerular capillaries and a high serum cholesterol. Bing and Starup [25] have shown that the excretion of cholesterol in renal disease runs parallel to the excretion of creatinin, urea and albumin. By increasing the permeability of the glomerular capillaries (uranium nitrate) with simultaneous experimental hypercholesteremia, Weltmann and Biach [26] and Richeri [27] produced lipoid changes in the renal tubular epithelial cells. Similarly, Korányi and Hámori [28, 29] showed again that injury of the glomerular capillaries makes the glomerulus permeable for serum albumin. It is this increased permeability of the glomerulus which is essential and primary in bringing about secondary morphologic tubular changes such as colloid droplets and lipoid droplets.

Conclusions

Colloid droplets in the lining cells of renal tubules of urodeles are due to absorption and storage of protein from the tubular lumina. "Natural" colloid droplets are not infrequently encountered in the "open" nephrons of urodeles as a result of the passage of protein-containing peritoneal fluid through the nephrostomes into the kidneys.

Intraperitoneal deposition of various protein-dye compounds, including homologous species proteins, is followed by the appearance of colored colloid droplets in the cells of the tubules of the "open" nephrons; their appearance was first observed 2 days after injection and they were still present 328 days afterwards.

In general, the size of the colloid droplets depends on the time elapsed since they were formed: at first they are large and occupy the supranuclear zone of the cytoplasm, but later they move towards the base of the cells where they become smaller.

No histologic evidence of splitting or digestion of colloid droplets within the renal epithelial cells could be detected. Partial reduction of colloid droplets is due either to desquamation of entire cells or to discharge of particles from tubular lining cells through tiny pores in the basement membrane of tubules, after which these are taken up by phagocytic stroma cells.

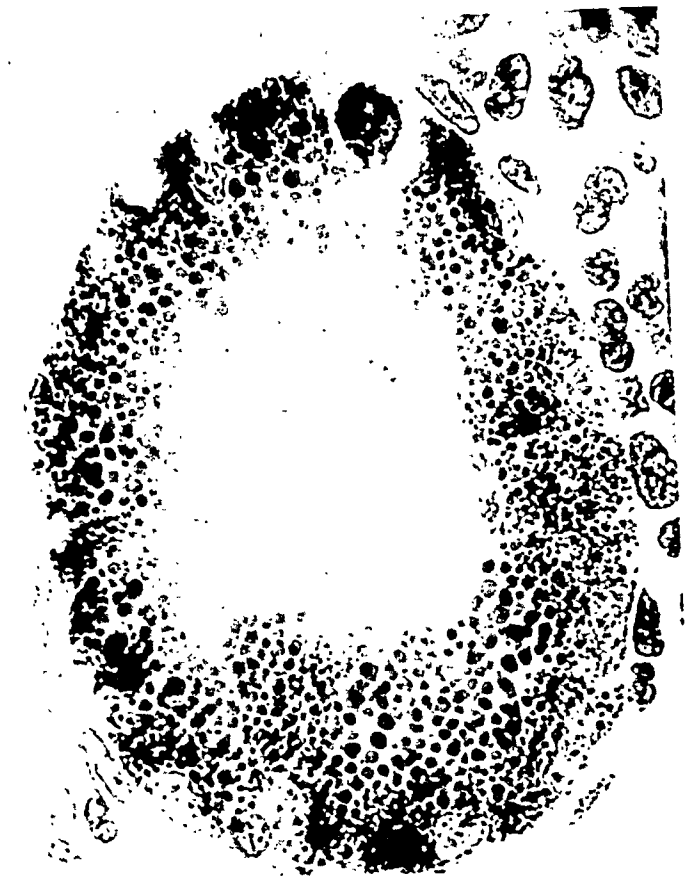
No specific site of storage within the tubules of proteins of various molecular sizes could be demonstrated; all proteins are absorbed and stored in the lining cells throughout the entire length of the "open" nephrons. Fats and lipids deposited in the peritoneal cavity of urodeles are phagocytized and stored in the tubules of the "open" nephrons in the same manner as proteins.



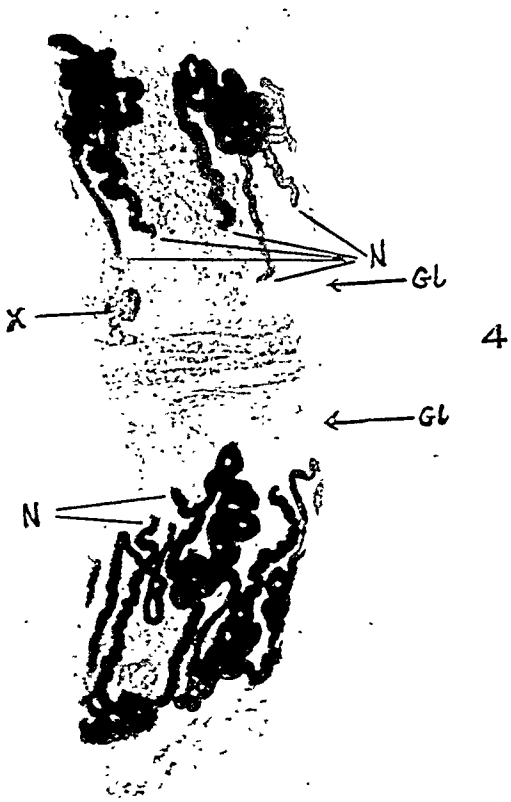
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DESCRIPTION OF PLATES

PLATE 161

FIG. 1. *Amblystoma punctatum.* Male. Four intraperitoneal injections, each of 0.5 cc. casein-R salt solution; 10 days after the first injection. The "open" nephrons are conspicuous due to the presence of casein-R salt in the tubular lining cells. N = nephrostome, W = wolffian duct. × 40.

FIG. 2. *Necturus maculatus.* Male. Four intraperitoneal injections, each of 0.5 cc. necturus serum albumin-R salt, 14 days after the first injection. The single segmental "open" nephron is clearly defined by the presence of albumin-R salt in the tubular lining cells. N = nephrostome, W = wolffian duct. × 15.

FIG. 3. *Necturus maculatus.* Male. Four intraperitoneal injections, each of 0.5 cc. dog serum globulin-R salt, 20 days after the first injection. Large, pale colloid droplets are seen in the supranuclear zone, and small, dark granules are present in the basal portion of the lining cells of an "open" tubule. × 460.

FIG. 4. *Necturus maculatus.* Male. Four intraperitoneal injections, each of 0.5 cc. egg albumin-R salt, 18 days after the first injection. Segment of kidney cleared according to the method of Spalteholz. The "open" tubules are clearly defined throughout their entire length due to the presence of egg albumin-R salt within the tubular lining cells. Gl = lines parallel to midline where glomeruli are located, some of which are faintly visible. X = dye-protein in glomerular space regurgitated from the tubule. N = junction of nephrostomial canal and "open" tubule. × 10.



Fig. 5



Fig. 6



Fig. 7



Fig. 8

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FIG. 5. *Necturus maculatus*. Male. Four intraperitoneal injections, each of 0.5 cc. necturus serum albumin-R salt, 60 days after the first injection. Colored granules and droplets are present in the basal portion of the lining cells of the "open" tubules; untinted "natural" colloid droplets occupy the supranuclear zone of the cytoplasm. × 460.

FIG. 6. *Necturus maculatus*. Male. Four intraperitoneal injections, each of 0.5 cc. dog serum globulin-R salt, 20 days after the first injection. Distension of lining cells of "open" tubules by colloid droplets with rupture of cell membrane and desquamation of entire cells into the lumina of the tubules. × 230.

FIG. 7. *Necturus maculatus*. Male. Four intraperitoneal injections, each of 0.5 cc. dog serum globulin-R salt, 87 days after the first injection. Dark red colloid droplets and granules are seen in the lining cells of the tubules and in phagocytes of the stroma outside the tubules. BM = basement membrane of "open" tubules. × 1050.

FIG. 8. *Necturus maculatus*. Male. Four intraperitoneal injections, each of 0.5 cc. dog serum globulin-R salt, 87 days after first injection. Extrusion of colored granules from a lining cell of the tubule through an opening in the basement membrane into the stroma where the material is taken up by a phagocyte. BM = basement membrane, P = pore in the basement membrane. × 1050.

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PLATE 163

FIG. 9. *Necturus maculatus*. Male. Five intraperitoneal injections, each of 0.5 cc. of human serum, case of nephrosis, 35 days after the first injection. Paraffin section stained by hematoxylin and eosin. Large, vacuolated lining cells of "open" tubules. A few small colloid droplets are seen along the inner border of the tubules. × 230.

FIG. 10. *Necturus maculatus*. Male. Five intraperitoneal injections, each of 0.5 cc. of human serum, case of nephrosis, 35 days after the first injection. Frozen section stained with scharlach R and hematoxylin. Lipoid-containing casts in lumina of "open" tubules and lipoid droplets in lining cells. × 230.

FIG. 11. *Necturus maculatus*. Male. Five intraperitoneal injections, each of 0.5 cc. of human serum, case of nephrosis, 35 days after the first injection. Frozen section stained with scharlach R and hematoxylin. Picture taken under the polarizing microscope. Doubly refractive cholesterol crystals in the tubular lining cells. × 230.

EFFECTS OF CENTRIFUGATION ON INTRANUCLEAR INCLUSIONS PRODUCED BY SUBCUTANEOUS INJECTIONS OF ALUMINUM OXIDE *

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By a program of work begun in 1935, we have sought to obtain information on the nature of intranuclear inclusions produced by viruses. The ultracentrifuge developed by Beams and Pickels [1, 2] has been used in all of these studies. It has been learned that there is a difference in relative specific gravity among inclusion bodies produced by various viruses. Herpes virus produces inclusions which are lighter than either the nucleoplasm or the basichromatin and oxychromatin.[3] Herpes is the only example of a granular type of inclusion body thus far investigated. However, several of the amorphous, nongranular types of inclusion bodies have been studied. In the submaxillary gland disease of guinea pigs the order from the lighter to the heavier pole of the centrifugated nucleus is nucleoplasm, inclusion body and a mixture of oxychromatin and basichromatin.[4, 5] In fox encephalitis, the sequence proves to be similar except that the inclusion body consists of two parts; an original basophilic center and a mantle of oxychromatin.[6]

The present investigation on intranuclear inclusions produced by chemicals represents further effort to identify and distinguish oxychromatin on the basis of its reaction when centrifugated, and when this has been accomplished to apply this information to acidophilic inclusions of virus origin.

Numerous investigators (Luger and Lauda,[7] Lee [8] and Blackman [9]) have produced an amorphous, nongranular type of intranuclear inclusion by introduction of various heavy metals, but in all of this work there is a possibility that the chemical used merely stimulated a latent virus which then made itself evident through the presence of an inclusion body. This alternate interpretation was removed, however, by the work of Olitsky and Harford [10, 11] when they tested, by passages and other means, the inclusions produced after subcutaneous injections of aluminum, iron, carbon and other substances. We employed their technic for injection of aluminum oxide and obtained similar intranuclear inclusions. Centrifugation of these inclusions has demonstrated a reaction similar to that of other amorphous, nongranular inclusions and has offered further evidence that this type of inclusion, when it

* Aided by a grant from the Research Council of Iowa State College. This aid supplemented facilities made available at the Veterinary Research Institute.
Received for publication, January 29, 1942.

except that masses of basichromatin aggregate into clumps around an acidophilic inclusion body (Fig. 1). Were other inclusion-bearing cells absent from the preparation, one would probably consider the central mass to represent a plasmosome nucleolus, surrounded by aggregated chromatin masses. The combined structure would be called an amphinucleolus.* With increasing age the small discrete basichromatin granules scattered over the linin network tend to aggregate into larger clumps and these move toward the nuclear membrane. In so doing they disappear from the linin network and the spaces of the network become larger. Chromatin granules still adhere to the surface of the acidophilic chromatin body (Fig. 2). In this particular nucleus the inclusion body was smaller than in the earlier stage; this confirms what has been emphasized in studies on virus-formed inclusions, that the size of the inclusion body is not necessarily a measure of its age, and that the degree of nuclear reaction is a more reliable criterion.[5, 6]

The process of margination continues, as well as coalescence of chromatin granules to form larger clumps. Frequently flecks of basichromatin still remain attached to the inclusion body after most of it has completely marginated, and ultimately all of it leaves the inclusion body. There still remain for a while, however, strands of the linin network, which extend across the halo surrounding the inclusion body (Fig. 3). The linin threads become less abundant and finally disappear, leaving an amorphous, nongranular inclusion body surrounded by a halo of nucleoplasm (Fig. 4). At such a stage of development the picture is morphologically identical with the nongranular type of inclusion body so frequently described in the literature as the sequela of a virus infection.

It has been difficult in these preparations to draw a sharp line between effects due to the formation of the inclusion body and the more generalized death sequence brought about by the chemical injected because the very conspicuous death reaction has frequently masked the other. In many nodules examined every nucleus of every type of cell present was a small pyknotic sphere. But on the basis of previous work it is suggested that the margination reaction is a response to the presence of the inclusion body and that the clumping of the chromatin is part of the sequence which may lead to death of the cell. Clumping in itself, however, does not predestine the death of the cell, for ample evidence has been offered that the chromatin of the nucleus may be contracted to a small, dense mass and then return to the normal dis-

* Olitsky and Harford [10] frequently use the term nucleolus. From the text it is evident that it is the karyosome or basichromatin type which is meant. They do not discuss the possible relationship of plasmosome nucleolus and inclusion body. They did note that amphinucleoli are not common, which agrees with our findings.

accompanies a virus disease, probably is composed in part or entirely of oxychromatin.

Materials and Methods

Injections of aluminum oxide, aluminum hydroxide, ferric hydroxide and carbon were tried. The best results were obtained with aluminum oxide and all of the data recorded are based on use of this chemical. In the early attempts a 1 per cent suspension in saline solution was used, but this proved so destructive to the infiltrating cells that the lower limit which Olitsky and Harford [10] recommended, namely, 0.75 per cent, was used thereafter. Even this concentration, in our hands, produced more destructive effects than we expected to find after examination had been made of a slide which was sent to one of us (A.M.L.) several years ago by Dr. Olitsky.

Various subcutaneous areas were injected: back, side, abdomen, axilla and groin. Best results were obtained when the nodules were removed from 17 to 23 days after injection. Several stains were tried and the only one which gave good differentiation was phloxine-methylene blue, as recommended by Olitsky and Harford.[10] Azure-eosin proved unsatisfactory on this material.

The ultracentrifuge employed was constructed according to directions given by Beams and Pickels.[2] The air pressure varied from 93 to 120 pounds and the maximum speed as determined by a Galton whistle was 3400 revolutions per second. The range in centrifugal force was from about 300,000 to 550,000 times gravity. Half of the excised nodule was placed in the centrifuge and half in a dish of Locke's solution. When centrifugation was completed both pieces were fixed in Zenker's fluid with 5 per cent acetic acid added.

Observations

It has been emphasized in earlier publications that in order to analyze the action of a virus upon a cell, it is necessary to distinguish between the reaction of the nucleus to the presence of an inclusion body and that due to the generalized destructive action of the virus or foreign agent which leads to necrosis of the cell. These two reactions proceed independently and the only effect which the latter may have on the former is to cut short the sequence of nuclear reactions so that the full response to the aging of the inclusion body is not obtained.

The Process of Inclusion Formation

Early nuclear reactions were not abundant in our material; when observed, however, the distribution of chromatin and the structure of the linin network is quite similar to that found in the normal nucleus

process of centrifugation? Guyer and Claus [14, 15] have contributed information in regard to these questions by implanting tissues in which the chromatin had been thrown entirely to one pole and have found that the implant survived. Beams and King [16, 17] followed the development of Ascaris eggs while they were centrifuged at 150,000 times gravity for 4½ days. They conclude, ". . . . that the killing of cells by the present methods of centrifugation is usually due to mechanical distortion or disruption (prevented in Ascaris eggs by the presence of a very resistant shell) rather than to a disturbance of the spatial relationship of their molecular parts."

Our approach to this problem has been a cytological one. Some nodules were centrifugated for 2 hours instead of the usual hour, and in this material every nucleus was clearly dead (Figs. 18 to 20). The cytological appearance was so much in contrast to the usual appearance with which we have been dealing that it increased our confidence that the typical configurations after centrifugation were not death artefacts. The killed nuclei were homogenous with no indication of the usual nuclear structure or inclusion bodies and also no indication of stratification due to centrifugating (Fig. 20). Some nuclei which had not yet completely developed the full death picture showed some evidence of stratification (Fig. 19), and a very few were found which had the appearance shown in Fig. 18. This resembles a centrifugated cell which is still living, except that even here there was evidence of laking of chromatin and loss of staining affinity. It is evident from the few nuclei which showed centrifugation effects that probably all of them had had this appearance before they were killed. Therefore, following death, the liquefied (?) contents became uniformly dispersed throughout the nucleus. It is not known whether the dispersion takes place while centrifugation is in progress or in the minute or less after centrifugation, before the fixative penetrates the cells.

Discussion

Are inclusion bodies produced by aluminum oxide and other metals identical with the amorphous or dropletlike inclusion bodies associated with virus diseases? Olitsky and Harford [10, 11] are of the opinion that they are identical and our own work confirms this opinion. The acceptance of this opinion forms a reference point against which previous conclusions based on centrifugation studies may be tested. There is little doubt in the case of aluminum oxide stimulation that the inclusion body is derived from the oxychromatin which is a normal constituent of the nucleus. It can be traced back to what would generally be regarded as a hypertrophied plasmosome and then by a gradual loss of

persed state without any apparent ill effect.[12, 13] However, when the nuclear membrane begins to shrink and there is a marked loss of nucleoplasm, what is probably an irreversible death change has set in and will lead to a pyknotic nucleus. Typical steps in the process are shown in Figures 5 to 8. During the process there seems to be very little fundamental change affecting the basichromatin or the inclusion body. It seems to be limited almost entirely to the loss of nucleoplasm, although, during the shrinking process, there is frequently observed a tinging of the nucleoplasm as if some of the chromatin had gone into solution.

Centrifugation of Inclusion-Bearing Cells

It is obvious that in the centrifugated nuclei the exact stage of nuclear reaction will be difficult to estimate because the basichromatin is concentrated during the process into a more or less compact mass at the centrifugal pole. However, one can break up the continuous process described in the preceding section into about three main divisions: early stage, adherence of basichromatin to the inclusion body; middle stage, absence of basichromatin on the inclusion body but no evidence of nuclear contraction (linin fibers may or may not extend from the inclusion body to the nuclear membrane); and last stage, loss of nucleoplasm and contraction of the nuclear membrane.

A few examples of the early stage have been found in the centrifugated preparations. They show abundant linin fibers stretched across the nucleus. The stage of nuclear reaction in Figure 9 is probably similar to that in the uncentrifugated nucleus shown in Figure 2. The next stage shows clearly the relative specific gravity of the three substances. The nucleoplasm is lightest, the inclusion next and the basichromatin is the heaviest (Figs. 10, 11, 12 and 14). There was no evidence of oxychromatin (other than that which forms the inclusion bodies) separating from basichromatin.

The last stage is represented by Figures 13, 15, 16 and 17. The nucleus in Figure 13, before centrifugation, probably corresponded quite closely to that shown in Figure 5 and illustrates an early stage in the shrinkage process. Loss of nuclear sap and shrinkage of the nuclear membrane does not change the relative specific gravity of the inclusion body and basichromatin (Fig. 15). Figures 16 and 17 were included because they show how the centrifugated nucleus may be turned at right angles when it strikes a barrier, in this case the cell wall of the enclosing giant cell.

The question naturally arises as to how much damage is being done to the cell by the process of centrifugation. Is the cell killed first and then the material stratified, or is the cell still alive at the end of the

probable sequence in the development of inclusion bodies and in the reaction of the nucleus which accompanies it.

(a) The earliest stage is the appearance of an amphinucleolus. The plasmosome portion becomes the future inclusion body.

(b) The chromatin, which in early stages was adherent to the inclusion body (plasmosome), leaves it and moves to the margin. Across the resulting halo are strands of the linin network.

(c) These break down and the inclusion body assumes an appearance identical with the amorphous or dropletlike inclusion body characteristic of some virus diseases.

(d) The last stage is necrosis which is made evident by shrinkage of the nuclear membrane and loss of nucleoplasm until all that remains is inclusion body surrounded by basichromatin and a nuclear membrane.

3. Centrifugation of these stages leads in each case to the same conclusion; namely, that the nuclear sap is the lightest substance, that the inclusion body is heavier and that the basichromatin is heaviest.

4. Comparison of these results with those from other centrifugation and cytological studies leads to the conclusion that oxychromatin is the material from which the acidophilic portions of the amorphous type of inclusion body, such as that found in submaxillary gland disease of the guinea pig and in fox encephalitis as well as those produced by heavy metals, are derived.

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adherent basichromatin, which moves to the margin, it becomes an inclusion body identical with the amorphous type of virus origin. Oxychromatin has a lower specific gravity than that of basichromatin, which fact was noticed first in the noninclusion-bearing cells of the fox. This agrees with results of the present study, and just as the amorphous inclusions produced by aluminum oxide come to lie on the centripetal side of the basichromatin so do the inclusions of submaxillary gland disease of the guinea pigs and of fox encephalitis stratify to similar positions. It is true that in these two virus diseases the inclusion bodies could not be traced back to a plasmosome nucleolus; in fact when one was present it was pushed to one side and marginated with the karyosome nucleolus which surrounded it. Nevertheless, in fox encephalitis the acidophilic material of the inclusion body arose by a generalized separation from basichromatin. Another point of similarity between amorphous inclusions of nonvirus and virus origin is their union with linin fibers in early stages of development and their separation later. A point of difference between the two is the fact that chromatin adheres to the inclusion (plasmosome nucleolus) of nonvirus origin when it is in its early stage of development, whereas in fox encephalitis and submaxillary gland disease it returns to join the oxychromatin inclusion body only in its late stages. This may prove to have more significance than it seems to have at the present time. In both, however, centrifugation fails to separate basichromatin when it is adhering to an inclusion body.

In the program of investigation thus far, the "granular type" of inclusion (herpes simplex in the rabbit cornea) has proved to be definitely different from the amorphous type. It would be especially interesting to learn whether pseudorabies virus and B-virus, when centrifugated, would react as does that of herpes. Also it would be helpful to know if a "particulate type" of virus inclusion (yellow fever) would behave like an amorphous or a granular type. It would probably help to answer the question whether the particulate type of inclusion is really oxychromatic degeneration as described in numerous publications.*

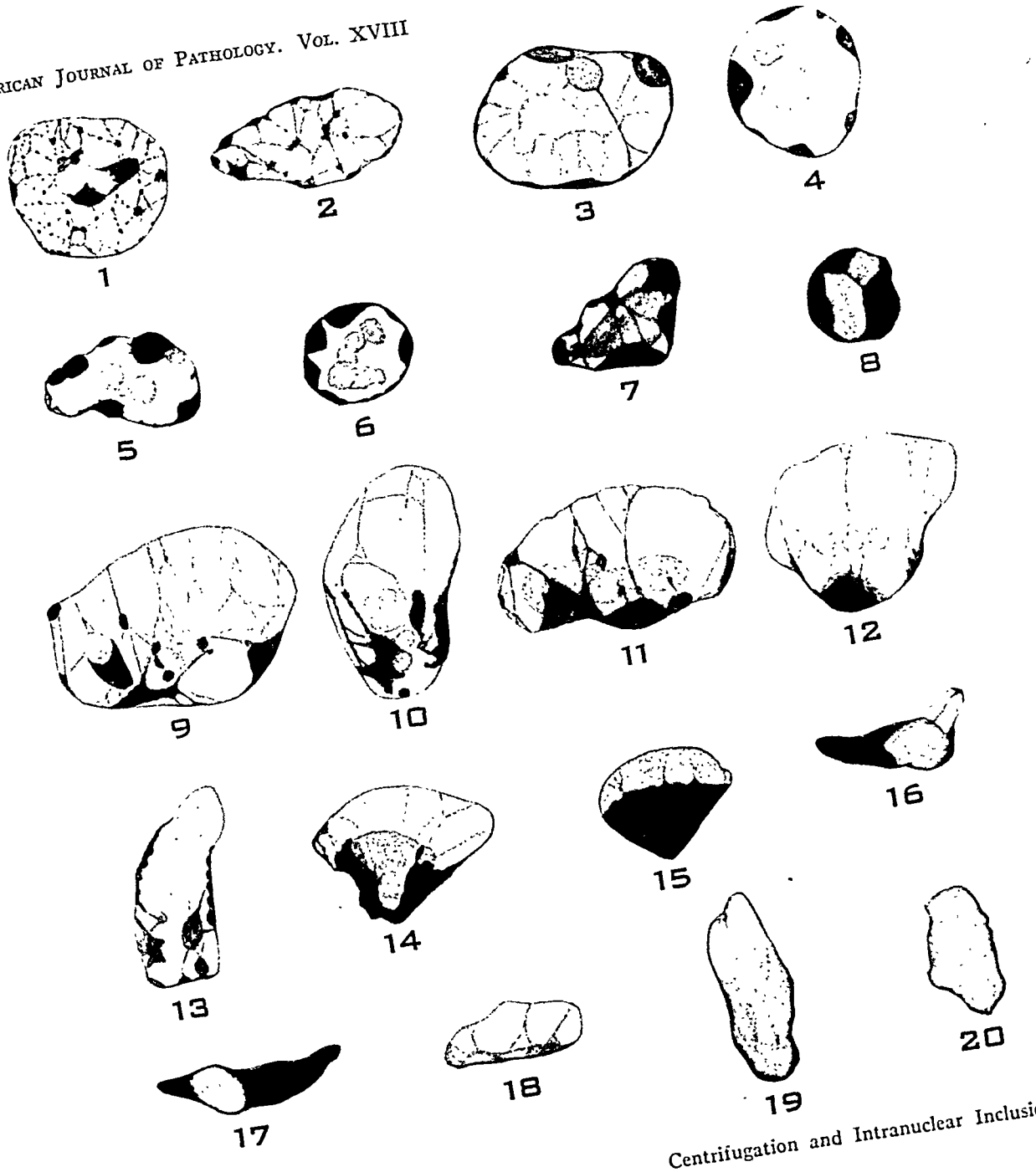
Summary and Conclusions

1. Experiments described by Olitsky and Harford on the production of intranuclear inclusions by subcutaneous injection of aluminum oxide have been repeated and their results confirmed.

2. The affected cells have been arranged in what is considered the

* Inclusions produced by pseudorabies, B-virus and panleukopenia of cats have been centrifugated since this paper went to press. It is evident from experiments thus far available that the type of tissue has an effect upon behavior of the inclusions during centrifugation.

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Centrifugation and Intranuclear Inclusions

Birch and Lucas

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DESCRIPTION OF PLATE

PLATE 164

All nuclei were drawn with a camera lucida at an optical magnification of 2000x and a projection magnification of 3200x. There has been no reduction in reproduction. All figures are of nuclei of cells which infiltrated nodules resulting from the subcutaneous injection of aluminum oxide in the guinea pig. All nuclei were stained with phloxine-methylene blue. Nuclei in Figures 1 to 8 were not centrifugated. Nuclei in Figures 9 to 20 were centrifugated at forces which varied during the centrifugation process from 300,000 to 550,000 times gravity. Those in Figures 9 to 17 were centrifugated for 1 hour and those in Figures 18 to 20, for 2 hours. All drawings of centrifugated nuclei are oriented so that the centrifugal pole is directed toward the bottom of the plate.

For description of the individual nuclei the reader is referred to the text.

PATHOLOGIC ALTERATIONS IN SURFACE RELATIONSHIPS AND MORPHOLOGY AT THE HUMAN SYNAPSE *

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INTRODUCTION

Anatomically, the term "synapse" refers to the contiguous components in a succession of neurons. From the fact that the transfer of an impulse across the synapse is unidirectional, the structure may be thought of as having an afferent, or distributing, component and an efferent, or receptive, component. The receptive structure includes the cell body, its dendritic processes and the root of the axon. In many locations in the central nervous system all three of these cellular areas act in a receptive capacity (prominent in Purkinje cells of the cerebellar cortex and in the lateral groups of final-common-path cells of the spinal cord). In other locations (nucleus dorsalis) the cell body and the root of its axon appear to be the principal receptive structures, with the dendrites playing little part. In still other regions (substantia gelatinosa Rolandi) the dendrites are described as the only receptive elements. The afferent or distributing component in each case includes the fibrillar axonic ramifications and the terminals (boutons) which bear on the receptive structures in question. The complexity of the afferent limb of the synapse appears to vary with the functional attributes of the efferent limb. Probably a great many distinct sources exist for supplying bouton connections to the final-common-path cells of the spinal cord. On the other hand, in some internuncial chains, the conterminous parts may represent relatively linear successions.

That these synaptic relationships may be disturbed under pathologic or experimental influence seems probable, although the demonstration of such changes has been subject to question. The use of morphologic or numerical changes in peripheral nerve endings as acceptable criteria of pathologic or experimental influence on the associated nerve cells or fibers is well established (Jackson,[1] Cajal,[2] Lawrentjew,[3] Gibson,[4] Carey,[5] Speidel [6]). Although one might reasonably expect similar accord with regard to central synaptic effects, nothing of the sort has appeared. Since Hoff's introduction of the bouton method as a means of establishing tract-nuclear relationships, a great many controversial reports have been presented (Hoff,[7, 8] and Barnard [9]). Probably the

* Read in part before the American Association of Neuropathologists, Atlantic City, N.J., June 9, 1941.
Received for publication, March 9, 1942.

less of age (after 1.5 years), provided certain limitations in the technic are observed and the same regions, including similar cell groups, are compared. The percentages of regular, thickened and granular boutons (forms), however, vary greatly from case to case and from cell column to cell column in the same material. In general, granular forms occur in relatively greater number in persons of middle age or older. It has been shown further that technic and autolysis are of little importance in accounting for the demonstrable variations in human bouton morphology.

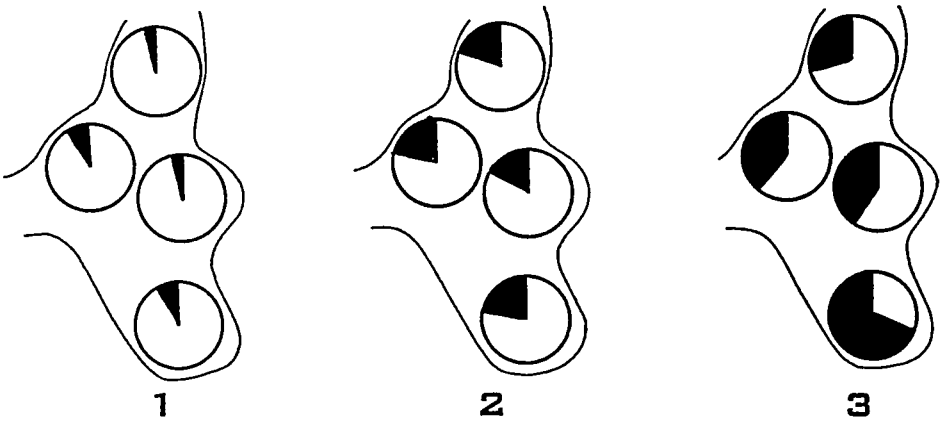
Of the influences of recognized potency in causing damage to the central nervous system which are operative in most cases that come to autopsy, the following have been mentioned: arteriosclerosis, inanition, intoxication, fever, dehydration, systemic infection and additional agonal correlatives. The material examined in the present study has been subject, as well, to lesions of the central nervous system which were clinically manifest. The immediate problems in connection with this material are (1) the determination of the manner in which, and the extent to which, surface relationships at the human synapse might be disturbed in neuropathologic involvements, and (2) the identification of a morphologic index of pathologic influence. An understanding of both numerical and morphologic variables is considered essential to the interpretation of findings in specific cases to be described. The observations on the neuropathologic material employed in the present study will be considered in the following sequence: (1) surface area of the receptive component of the synapse; (2) number of terminals and contact surface at the synapse; (3) alteration in number of terminals (related to pathology, regional differences, autolysis, technic, age); (4) alteration in morphology of terminals; (5) influence of the fundamental nature of the pathologic process on both number and morphology of terminals; (6) influence of duration of the pathologic process; (7) the factor of distance between the lesion and the alterations observed; (8) progressive and retrograde effects at the synapse; (9) individual case data. Since the pathologic changes of the receptive component of the synapse (chromatolysis, fragmentation, shrinkage, nuclear change) are fairly well established, no special effort has been made to review this field. The observations in the present study are primarily on the distributing components of the synapse, and, of these, particularly on the nerve terminals, or boutons.

Method and Material

The spinal cords used in the present study were removed at autopsy by the Kernohan [12] method and the sections treated with Cajal's silver

most general criticisms of the acceptability of the procedure have been on the bases of (1) technic and (2) inadequacy of control. It must be admitted that these criticisms are most applicable to the human material which is employed in the present study. Nevertheless, in view of the current interest in central synaptic phenomena, it has seemed permissible to proceed by anticipating the criticisms as far as possible with quantitative methods which establish a predictable synaptic morphology with the standard technic used.

In a recent report [10] the boutons (nerve terminals) of the human spinal cord, appearing in block silver preparations, are described in five primary morphologic groups (types)—small loops, large loops, filamented loops, fibrillated bulbs, and opaque or granular masses—and three subgroups (forms)—regular, thickened, and granular. A schema of this classification is presented as the legend to Figures 1, 2 and 3 with references in appropriate columns to the morphologic "types" and "forms" of boutons appearing in the photomicrographs. Because of the relative unimportance (numerically) of regular and thickened filamented loops, the publication of additional pictures to illustrate these structures seems unwarranted. A quantitative study [11] of the nerve terminals in 26 cases with no clinically demonstrable pathology of the central nervous system has revealed that the same proportions of the various morphologic types of boutons occur from case to case regard-



TEXT-FIGURE 1. This schematic diagram of a hemisection of the gray matter of the thoracic cord shows the average percentages of granular forms (blackened sectors) of terminals ending in relationship to the cells of the principal nuclear groups in non-neuropathologic cases under 30 years of age. The posterior sector in the gray matter represents the boutons around cells of the sensory and internuncial groups; the medial sector, of the nucleus dorsalis; the lateral intermediate sector, of the intermediolateral column; and the anterior sector, of the somatic efferent columns.

TEXT-FIGURE 2. A diagram corresponding to Text-Figure 1, but for nonneuropathologic cases over 30 years of age.

TEXT-FIGURE 3. The percentages of granular forms of terminals around cells of the principal nuclear columns in a thoracic field of case H108, poliomyelitis.

TABLE I—*Continued*

Case	Age	Autolysis time	Formalin time	Duration of lesion	Diagnosis
H74	3 mos.	12 hrs.	20 hrs.		Hydranencephalus
H75	28 yrs.	24 hrs.	18 hrs.	1 da.	Fracture of cervical vertebrae
H76	67 yrs.	2 hrs.	22 hrs.	1 da.	Fracture, T9 and T10
H77	70 yrs.	16 hrs.	22 hrs.		Cerebral arteriosclerosis
H81	75 yrs.	3 hrs.	21 da.		Cerebral arteriosclerosis
H82	20 yrs.	3 hrs.	20 hrs.	10 da.	Subacute bacterial endocarditis; clinical encephalitis
H83	62 yrs.	5 hrs.	15 mos.	7 wks.	Fracture of 5th cervical vertebra
H84	52 yrs.	13 hrs.	15 mos.	3 wks.	Purulent meningitis
H85	15 yrs.	3 hrs.	16 mos.	3 wks.	Purulent meningitis
H86	45 yrs.	7 hrs.	19 mos.	7 da.	Fracture and fat embolism
H87	61 yrs.	27 hrs.	19 mos.		Luetic meningeoencephalitis (general paresis with tabes)
H88	20 mos.	3 hrs.	6 yrs.		Avitaminosis A; necrosis of brain and cord
H89	39 yrs.	24 hrs.	13 yrs.	10 mos.	Actinomycotic myelitis
H90			5 yrs.	2 da.	Poliomyelitis
H91	14 yrs.	2 hrs.	6 yrs.	7 da.	Fracture of cervical vertebrae
H92	55 yrs.		12 yrs.		Subacute necrotic myelitis
H93	28 yrs.	4 hrs.	4 yrs.	7 da.	Purulent meningitis
H94	23 yrs.	2 hrs.	5 yrs.	12 da.	Poliomyelitis
H95	10 yrs.	2 hrs.	5 yrs.	3 da.	Polioencephalomyelitis
H96	46 yrs.	7 hrs.	8 yrs.	7 wks.	Vitamin D deficiency(?); degeneration of anterior horn cells
H97	36 yrs.	2 hrs.	7 yrs.		Malnutrition
H98	30 yrs.	2 hrs.	10 yrs.	30 da.	Meningeoencephalomyelitis
H99	28 yrs.	3 hrs.	10 yrs.	27 yrs.	Diaphragmatic hernia; old poliomyelitis
H100	28 yrs.	5 hrs.	12 yrs.	16 da.	Cord necrosis
H101	25 yrs.	2 hrs.	6 yrs.	5 da.	Meningeoencephalomyelitis (poliomyelitis)
H102	39 yrs.	2 hrs.	15 yrs.	1 mo.	Lues; resolving purulent meningitis
H103	37 yrs.	10 hrs.	11 yrs.	28 da.	Cord necrosis
H104	48 yrs.	9 hrs.	5 yrs.		Pigment degeneration and atrophy of ganglion cells
H105	54 yrs.	2 hrs.	17 yrs.		Pellagra; clinical encephalitis
H106	14 yrs.	9 hrs.	10 yrs.	5 da.	Poliomyelitis with involvement of dorsal root ganglion cells
H107	10 mos.	2 hrs.	10 yrs.	4 da.	Hemorrhagic encephalomyelitis
H108	16 mos.	6 hrs.	5 yrs.	12 da.	Poliomyelitis
H109	8 yrs.	2 hrs.	5 yrs.	2 wks.	Postmeasles encephalomyelitis
H110	22 yrs.	3 hrs.	6 yrs.	3 da.	Polioencephalomyelitis
H111	52 yrs.	3 hrs.	6 yrs.	50 yrs.	Ulcerative colitis; peritonitis; ancient poliomyelitis
H112	9 yrs.	6 hrs.	12 da.	7 da.	Tetanus
H113	49 yrs.	12 hrs.	6 yrs.	? yrs.	General peritonitis; cerebellar atrophy
H114	59 yrs.		9 yrs.	1 yr.	Cerebellar atrophy
H115	74 yrs.		3 yrs.	18 yrs.	Cerebellar atrophy

nitrate, modification 4 (after Barr [13]). Since the earlier reports, considerable material has been used which has lain in formaldehyde from 1 to 17 years. Although unsatisfactory in a few cases, some excellent preparations have been obtained from material fixed in formaldehyde for long periods. In checking the nature and distribution of lesions, routine methods have been employed, including hematoxylin and eosin,

TABLE I

Essential Data from Case Histories

Case	Age	Autolysis time	Formalin time	Duration of lesion	Diagnosis
H1	45 yrs.	4 hrs.	25 hrs.	29 da.	Fracture at 4–5 cervical vertebrae
H3	65 yrs.	2 hrs.	26 hrs.	2 yrs.	Hodgkin's disease; generalized sepsis; old pontine infarct
H5	12 yrs.	3 hrs.	15 hrs.	6 da.	Toxic encephalomyelitis (Landry)
H6	58 yrs.	24 hrs.	19 hrs.	2 yrs.	Cerebral thrombosis with old encephalomalacia
H7	15 mos.	8 hrs.	70 hrs.	15 da.	Purulent meningitis
H8	41 yrs.	17 hrs.	22 hrs.	5 wks.	Left cerebral tumor
H9	57 yrs.	11 hrs.	21 hrs.	9 da.	Carcinoma of esophagus (inanition)
H11	61 yrs.	3 hrs.	14 hrs.	3 da.	Arteriosclerosis; suppurative pericarditis; uremia
H12	73 yrs.	7 hrs.	24 hrs.	4 da.	Cerebral hemorrhage
H16	36 yrs.	13 hrs.	10½ mos.	26 yrs.	Amputation of right leg (amyloidosis; uremia)
H17	39 yrs.	7 hrs.	22 hrs.	4 mos.	Metastatic brain tumor
H18	54 yrs.	13 hrs.	23 hrs.		Cerebral arteriosclerosis
H20	57 yrs.	6 hrs.	23 hrs.		Luetic meningitis
H23	70 yrs.	2½ hrs.	22 hrs.		Cerebral arteriosclerosis
H24	57 yrs.	13 hrs.	22 hrs.		Carcinoma of stomach (inanition)
H27	68 yrs.	11 hrs.	26 hrs.		Luetic meningitis
H29	52 yrs.	11 hrs.	23 hrs.	5 yrs.	Left cerebral tumor
H31	73 yrs.	5 hrs.	20 hrs.		Perforating duodenal ulcer; arteriosclerosis
H37	20 yrs.	2 hrs.	24 hrs.	2½ mos.	Brain tumor obstructing ventricles
H39	72 yrs.	7 hrs.	23 hrs.	5 mos.	Left leg amputation; diabetes mellitus; arteriosclerosis
H40	53 yrs.	15 hrs.			Diabetes; hypoglycemia
H41	27 yrs.	3 hrs.	17 da.	7 da.	Polioencephalomyelitis
H42	2½ yrs.	4 hrs.	28 hrs.	4 mos.	Tumor of right cerebellar hemisphere
H43	4½ yrs.	11 hrs.	21 hrs.	3 wks.	Postinfectious encephalomyelitis
H44	62 yrs.	4 hrs.			Diabetes; carcinoma of stomach
H45	51 yrs.	4 hrs.	39 hrs.	3 mos.	Right brain tumor (glioblastoma multiforme)
H47	23 yrs.	14 hrs.	39 hrs.	2 yrs.	Uremia; chronic glomerulonephritis; otitis media
H48	47 yrs.	13 hrs.		6 da.	Tuberculous pneumonia; chronic alcoholism
H49	41 yrs.	3 hrs.	13 hrs.	3 mos.	Metastatic brain tumor; old cord demyelination
H50	38 yrs.	5 hrs.	26 hrs.	1½ yrs.	Multiple myeloma with destruction of cord below T7
H51	16 yrs.	4 hrs.	40 hrs.	3 mos.	Seminoma of left testicle; uremia
H53	14 yrs.	12 hrs.	30 hrs.	37 da.	Purulent meningitis
H55	80 yrs.	3 hrs.			Cerebral arteriosclerosis
H56	54 yrs.	3 hrs.	32 hrs.	1½ mos.	Hemolytic streptococcic septicemia
H58	18 yrs.	3 hrs.	57 hrs.		Appendectomy followed by hyperpyrexia; pituitary dwarfism
H60	60 yrs.	14 hrs.	23 hrs.	13 da.	Tetanus
H62	53 yrs.	2 hrs.	24 hrs.	1 yr.	Right cerebral tumor
H64	79 yrs.	5 hrs.	30 hrs.	2 yrs.	Metastatic vertebral tumor (L_2–L_5) with damage to cauda
H70	79 yrs.	5 hrs.	20 hrs.	73 yrs.	Pneumonia; old poliomyelitis
H71	29 yrs.	3 hrs.	48 hrs.		Generalized miliary tuberculosis; tuberculous meningitis
H72	33 yrs.	3 hrs.	7 da.	1½ mos.	Left frontal brain abscess
H73	65 yrs.	8 hrs.	14 da.	3 wks.	Multiple sclerosis (?); old toxic myelitis(?); pernicious anemia(?)

and distributed in slices in the section examined. The total surface area appearing in the region, divided by the theoretical number of cells, gave an estimate of the surface area per cell. In measuring, peripheral dendrite, dendrite root, cell body and axon root fragments were differentiated. (No fragment under 2 μ in diameter was counted since no synaptic contacts on cell structures of less than this magnitude have been noted by me.) The estimates of cell surface area and the proportion of the total area contributed by cell processes in the various columns of the spinal cord are given in the first two columns of Table II.

The differences in cellular surface area among the various columns

TABLE II

Contact Surface Area and Number of Boutons per Cell

Nuclear column	Computed cell surface (μ^2)	Process surface as % of total	Computed boutons per cell	Contact surface (μ^2)	Per cent of cell surface engaged in contact
Dorsal sensory and internuncial	756	35%	97	321	
Intermediate sensory and internuncial	1,053	44%	152	515	55.03%
Ventral sensory and internuncial	510	27%	116	376	
Nucleus dorsalis	4,372	52%	348	1,354	30.97%
Intermediolateral	1,563	38%	122	397	25.40%
Posteromedian	2,063	56%	362	1,410	
Anteromedian	3,949	63%	675	2,637	48.50%
Anterolateral	20,143	81%	1,624	6,349	
Posterolateral	56,479	81%	3,990	15,593	

(510 μ^2 to 56,479 μ^2) appear to be far greater than would be imagined from inspection of cross section area of the cell bodies. It is interesting to note the enormous relative surface area (81 per cent) contributed by the processes of the lateral groups of final-common-path cells. The cell body area listed for cells of the nucleus dorsalis included the surface of the root of the axon which in this case amounted to 30 per cent of the total surface.

Lassek [14] has recently cited three conceptions of the significance of cell caliber in the motor cortex: (1) that cell size varies with the "size of the movement" it governs (Jackson [15]); (2) that cell size varies with the distance over which its impulse must travel (Lewis [16]), and (3) that cell size depends on the extent of the unit it supplies (Fulton [17]). That these factors apply as well to the cells of the spinal cord would seem evident from the known distributions of the efferent groups and the nucleus dorsalis. On the same basis the axon connections of the receptive and internuncial groups would probably be somewhat as follows: ventral group to efferent cells of the same side and possibly

cresyl violet, Marchi, Weigert, and Masson's trichrome stains. The boutons in certain locations, particularly in the cerebellar cortex, did not respond well to modification 4 and required staining with pyridine silver. In these instances the control blocks were handled in the same manner. Pyridine silver was also employed in the material from individuals under the age of 1 year with more success than attended the use of the usual method.

The method of counting and classifying the boutons and of determining their proportionate distribution has been described.[10] Where the procedure for cord nuclei was not applicable, as in cerebellar cortex and nuclei, methods were employed which will be described with the bouton distribution in the region. The procedures and methods of control will be explained in the treatment of each subdivision of the general observations.

The 81 cases examined in the present study are listed in Table I, together with essential data on prominent factors which might be expected to influence the bouton picture. It will be noted that the duration of the neuropathologic process could not be ascertained in many cases. In some of these the reason will be evident in the diagnosis, in others no information was available. It should be noted, too, that the duration of the neuropathologic processes does not necessarily correspond to the duration of the terminal illness, which in itself may be of considerable importance.

Observations

I. Surface area of the receptive component of the synapse

Preliminary to an inspection of altered conditions at the synapse it has seemed advisable to ascertain as closely as possible the normal surface relationships between the contiguous parts. In estimating the surface area of the receptive components of the synapse the following procedure has been employed: sections 10 μ in thickness were examined systematically under oil immersion, noting the number, linear dimensions, configuration and source of the nerve cell fragments in the various columns of the gray matter. The total cell surface area in each nuclear group in the section was computed by adding the surfaces of the cell fragments (computed individually by appropriate formulae fitting cones, spheres, cylinders, ellipsoids, or parts of each, depending on the configuration of the fragment in evidence). A linear estimate of total cell *body* length in the section of each column was computed as the product of the number of cell bodies cut times the depth of the section. This value divided by the average length of the cells of the location in question was taken as the theoretical number of cells cut

should probably be considered maximal rather than average for the technic used (comparison may be made with actual counts representing number per cell body in a previous report [10]).

Crude estimates of total synaptic surface relationships in the human cord are given in Table III. The estimated number of cells in each column is a linear multiple (based on length of column) of the average theoretical number of cells appearing in each section counted. The total receptive surface is computed from the surface-per-cell value in Table II. The computed total values for boutons and for contact surface are likewise based on the data of Table II.

TABLE III

Estimated Total Synaptic Surface Relationships in Cord

Nuclear column	Estimated no of cells	Estimated total cell surface ($mm.^2$)	Total no. of boutons	Contact surface ($mm.^2$)	Per cent of total by groups
Dorsal sensory and internuncial	1,820,000	1,375	176,540,000	646	
Intermediate sensory and internuncial	2,230,000	2,348	338,960,000	1,291	35.5
Ventral sensory and internuncial	1,390,000	708	161,240,000	389	
Nucleus dorsalis	188,600	825	65,632,800	247	3.8
Intermediolateral	1,023,000	1,599	124,806,000	399	6.1
Posteromedian	825,000	1,702	298,650,000	817	
Anteromedian	519,000	2,050	350,325,000	984	54.6
Anterolateral	72,000	1,451	116,928,000	696	
Posterolateral	40,000	2,260	159,600,000	1,084	
Total	8,107,600	14,318	1,792,681,800	6,553	

Assuming a measure of reliability for the data of Table III, it is interesting to note that approximately 18 per cent of the cells in all groups listed (those of somatic efferent groups) account for over 50 per cent of the total synaptic contact surface in the cord. It will be noted that a number of cord cell columns are omitted in Table III; these would, of course, increase the total values listed. The identification of the sources of terminals contributing to these synapses and a quantitative expression of the relative importance of these sources are problems of great importance in understanding spinal synaptic activity.

The remainder of the present paper outlines the procedures used in employing pathologic material in determining these relationships, based on alteration in number or morphology of afferent synaptic elements.

3. ALTERATION IN THE NUMBER OF TERMINALS AT THE SYNAPSE

Since errors in direct counts are magnified by transcribing the sample counts to a total synapse picture (as in Table III), it has seemed ad-

short homolateral intersegmental connections; intermediate group, long crossed and long homolateral intersegmental connections; and the dorsal group, shorter crossed and short homolateral intersegmental connections. The disproportion in cell size relative to its metabolic responsibilities, as evident in the surface area data of Table II, would suggest that additional factors are operative in dictating cell size. Of these, the provision of adequate receptive contact surface for varying complexities of afferent synaptic components would seem to be of prime importance. A comparison of the surface area on a cell of the nucleus dorsalis with that of a cell of the anterolateral group illustrates the point. The cell diameters in the two groups are approximately the same and the axons presumably travel about the same distance; but the surface area of a cell of the latter group is approximately four times that of a cell of the nucleus dorsalis (Table II). This suggests that cell size (surface) is perhaps more closely related to its receptive capacity than to its axon distribution.

A prominent exception to any of the above views relative to cell size is found in the constituents of the nucleus substantia gelatinosa Rolandi which are commonly described as second order neurons in the pain and temperature pathway. Their axons presumably pass to the opposite side and ascend in the spinothalamic tract—a fact which is entirely out of keeping with the size of these cells. It would seem necessary to view these cells, on the basis of size, either as very short internuncial elements or as unassigned by function. As reported previously, the synaptic relationships of these cells are obscure with the technics used in the present study.

2. NUMBER OF TERMINALS AND CONTACT SURFACE AT THE SYNAPSE

Estimates of the number of boutons ending in relationship to nerve cells (computations based on spherical shape of cell bodies) have been reported (Barr,[13] Minckler [10]). The present consideration is an effort to refine these estimates by basing the computation on more accurate determinations of cell surface [18] (Table II). The values listed in Table II (column 3) have been determined from the number of terminals counted on cell fragments, the average size of each, the optimal density of boutons on each part of the receptive component and the fraction of the available contact surface involved. These data are summarized in the last column as contact surface with an estimate of the percentage of the cell surface engaged in synaptic contact in the four main groups of cells (afferent and internuncial, nucleus dorsalis, intermediolateral column, and somatic efferent). Because of the selection of optimal preparations in determining bouton density on cell bodies, these data

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terminals per cell section (averages) and the range in the counts for the number of boutons and cell sections indicated are given for nine sections at four levels. These counts were from cases H6, H23, H31, H12 of the present series, and nos. 41, 42, 52, 56, 59 of the series previously reported.[11] These patients varied in age from 58 to 83 years; in time between death and autopsy, from 3 to 24 hours; in time in formalin from 19 to 33 hours. Arteriosclerosis was common to all. The suggestions from the table that the internuncial cells are relatively

TABLE V

Distribution of Counts on 19 Nonneuropathologic Sections (o) *and 92 Neuropathologic Sections* (x) *According to the Average Number of Terminals per Cell Section. Age Groups and Nuclear Columns are Not Differentiated*

Number of boutons per cell section	
0–1	x x x x x x x x x o
1–2	x x x x x x x o
2–3	x x x x x x x x x x x x x o o o o o
3–4	x x x x x x x x x x x x x x x x x x x o o o o o
4–5	x x x x x x x x x x x x x x x x x x x x o o o o o
5–6	x x x x x x x x x x x x o
6–7	x x x x x x x x o
7–8	x x
8–9	x x
9–10	x x
10+	x x

more receptive in the cervical enlargement and that the somatic efferent groups of the lower part of the cord have relatively more associations may be unfounded because of the small sample necessitated by selection of parallel cases.

Table VII compares the average number of boutons per cell section in two cases (H6, H18), autopsied 13 and 24 hours after death, with three cases (H23, H12, H3), autopsied 2, 2.5 and 7 hours after death. All were patients with arteriosclerosis, varying in age from 54 to 73 years, and all sections listed were from the thoracic region. The time in formalin varied from 19 to 26 hours. It seems apparent that no

TABLE IV

Average Number of Boutons per Cell Section Appearing in the Various Nuclear Columns in Nonneuropathologic and Neuropathologic Cases

	Afferent and internuncial			Nucleus dorsalis			Intermediolateral			Somatic efferent			Total averages		
	Cell sections	Boutons	Boutons per cell section	Cell sections	Boutons	Boutons per cell section	Cell sections	Boutons	Boutons per cell section	Cell sections	Boutons	Boutons per cell section	Cell sections	Boutons	Boutons per cell section
Nonneuropathologic (19 cases)	899	1947	2.17	329	1320	4.01	192	440	2.29	850	4388	5.16	2270	8095	3.57
Neuropathologic (78 cases)	3745	10033	2.68	1044	4820	4.26	840	1690	2.01	4071	24117	5.92	9700	40660	4.19

visable to leave the values as expressions of the actual observations (number of terminals per cell section) in the comparisons below. Since the main influence to be examined as instrumental in effecting change at the synapse was pathologic involvement of nerve elements, a general comparison of the average number of terminals per cell section found in neuropathologic and nonneuropathologic cords was the first consideration (Table IV). With the exception of the terminals adjacent to cells of the intermediolateral column, it would seem from Table IV that the number of terminals was increased under the influence of disease. These average values are subject to a great many controllable variables and depend primarily, as will be shown later, on selection of case types. A more descriptive picture of this comparison is the graph (Table V) of the average number of terminals per cell section in all nuclear groups. From this table it is evident that the averages in the two groups correspond fairly well but that the extremes (cases showing less than two or more than seven boutons per cell section) are peculiar for the most part to the neuropathologic group. This greater variability in each direction for neuropathologic cases persists in the number of terminals in individual nuclear columns.

In arranging Tables VI, VII, VIII and IX an effort was made to ascertain to what extent this demonstrated variability in bouton counts may be related to regional differences in the spinal cord, to autolytic changes, to differences in technic and to age. The cases in each comparison have been selected to eliminate, as far as possible, all variables except the one in question. In Table VI the number of

may be mentioned which may have a bearing on such a disposition. The younger group included four neuropathologic cases and two non-neuropathologic cases under 1.5 years, which age group has previously been described as poor material for demonstrating boutons.[10] In addition, the relatively greater proportion of high counts for neuropathologic cases in the younger group may be related to the nature of the pathologic processes, a large proportion of these being inflammatory (meningitis, myelitis).

TABLE VII

Comparison of Number of Boutons per Cell Section in Cases Subject to Autolytic Influence of More Than 13 and Less Than 7 Hours

	Sensory and internuncial			Nucleus dorsalis			Intermediolateral			Somatic efferent		
	Cell sections	Boutons	Boutons per cell section	Cell sections	Boutons	Boutons per cell section	Cell sections	Boutons	Boutons per cell section	Cell sections	Boutons	Boutons per cell section
More than 13 hours (2 sections)	100	318	3.18	53	283	5.34	29	79	2.72	99	764	7.71
Less than 7 hours (3 sections)	105	272	2.59	71	419	5.90	54	141	2.61	157	1001	6.38

A more adequately controlled comparison by nuclear columns in nonneuropathologic material (from cases in a previous report[11]) is shown in Table X, with three sections counted from cases 9 and 14 (ages 11 and 20) and three sections from cases 25, 27 and 32 (ages 46, 50 and 54). All sections were from the thoracic region, all were subject to less than 8 hours of autolysis and all were submitted to the same technic. No lesions of the central nervous system were evident in any of the cases. The disparity in the counts of endings around the cells of the nucleus dorsalis and the somatic efferent column would suggest

TABLE VIII

Comparison of Number of Boutons per Cell Section Counted in Same Case Subject to Different Periods of Treatment with Formalin

	Dorsal horn			Ventral horn		
	Cell sections	Boutons	Boutons per cell section	Cell sections	Boutons	Boutons per cell section
H39 Lumbar Formalin 23 hours	372	1518	4.08	264	1858	7.04
H39 Lumbar Formalin 1 year	231	892	3.86	24		

TABLE VI

Comparison of Number of Boutons per Cell Section in Cord Regions

	Sensory and internuncial				Nucleus dorsalis				Intermediolateral				Somatic efferent			
	Cell sections	Boutons	Av. no. per cell section	Range	Cell sections	Boutons	Av. no. per cell section	Range	Cell sections	Boutons	Av. no. per cell section	Range	Cell sections	Boutons	Av. no. per cell section	Range
Cervical (1 section)	49	94	1.92	1.5 to 2.9									55	327	5.95	3.0 to 8.7
Cervical enlargement (1 section)	71	217	3.06	2.4 to 3.8									100	541	5.41	4.3 to 6.6
Thoracic (4 sections)	144	396	2.75	1.6 to 3.7	82	482	5.88	4.6 to 7.0	59	168	2.85	2.5 to 3.0	178	1130	6.35	5.4 to 7.0
Lumbar enlargement (3 sections)	241	608	2.52	1.0 to 4.5	51	275	5.40	2.7 to 7.6	19	40	2.11	2.1 to 2.2	159	1171	7.36	3.3 to 9.6

boutons disappeared as a result of autolytic processes within the period indicated.

The only variable of any magnitude in the technic applied in the present study is the period of time during which the tissues lay in formalin. Counts from two blocks from the same region of H39, one prepared after 23 hours of immersion in formalin and one after 1 year, are listed in Table VIII. The slight difference in the two counts is not considered significant enough to indicate a diminution in the number of terminals due to the action of formaldehyde. Obviously, this one instance was a poor control for the entire series, but no additional means of checking the point with human material was available.

Of considerable interest in an analysis of this kind is the effect of age on the number of demonstrable terminals. That aging and its attendant wear have some effect on the morphology of boutons in nonneuropathologic cases has been reported.[11] The distribution of the counts in the present series by number of terminals per cell section is indicated in Table IX in two age groups, under and over 30 years. The small number of nonneuropathologic cases in the younger group prohibits any conclusions from these comparisons but certain factors

fundamental conceptions of the nature of alteration at the synapse are ascertainable only from group data. These are essential to the interpretation of data from individual cases to be described later.

The distribution of the boutons by "type" (Figs. 1, 2 and 3, and schema), expressed as percentages of the total number counted in 26

TABLE X

Comparison of Number of Boutons per Cell Section in Two Age Groups (Nonneuropathologic)

	Sensory and internuncial			Nucleus dorsalis			Intermedio-lateral			Somatic efferent		
	Cell sections	Boutons	Boutons per cell section	Cell sections	Boutons	Boutons per cell section	Cell sections	Boutons	Boutons per cell section	Cell sections	Boutons	Boutons per cell section
Under 20 years (3 sections)	164	382	2.33	188	401	3.40	77	206	2.67	147	920	6.26
Over 45 years (3 sections)	131	322	2.46	64	326	5.09	47	115	2.45	124	611	4.93

nonneuropathologic cases and in 20 cases with damage to the central nervous system are given in Table XI. These data indicate that the fundamental "types" of nerve terminal configurations are not disturbed under pathologic influence.

In Table XII the percentages of regular, thickened and granular "forms" (Figs. 1, 2 and 3, and schema) of boutons in the same two

TABLE XI

Comparison of the Proportionate Distribution of the Five "Types" of Boutons in Nonneuropathologic and Neuropathologic Spinal Cords

	Type 1 Small loops	Type 2 Large loops	Type 3 Filamented loops	Type 4 Fibrillated bulbs	Type 5 Opaque and granular bulbs
Nonneuropathologic (26 cases)	46.36%	31.04%	0.67%	19.68%	2.23%
Neuropathologic (20 cases)	45.49%	31.97%	1.01%	19.51%	1.95%

groups are compared. Approximately 12 per cent more granular forms and correspondingly fewer regular forms occurred in the neuropathologic cases. As in the comparisons reported for the effects of technic, autolysis and aging in nonneuropathologic cases,[11] the thickened forms occurred in constant proportion. Because of this consistency in the appearance of thickened terminals (representing increased silver affinity) these structures may not be considered an indication of pathologic change in the human spinal cord (see, however, Hoff[7, 8] and

that the former were increased and the latter diminished with age. Certain points which might clarify such a relationship will be introduced later in the discussion of spatial influence on terminal change.

It is concluded from the group data above that pathologic processes may effect either an increase or a decrease in the number of terminals

TABLE IX

Distribution of Total Counts Arranged in Two Age Groups

No. of boutons per cell section	Under 30 years	30 years and over
0–1	x x x o	x x x x x x
1–2	x x x o	x x x x
2–3	x x x x x o	x x x x x x x x o o o o
3–4	x x x x x o o	x x x x x x x x x x x x o o o
4–5	x x x x x x x	x x x x x x x x x x x o o o o o
5–6	x x x x	x x x x x x x x o
6–7	x x x x	x x x x o
7–8	x	x
8–9	x	x
9–10	x	x
10+	x	x

o = nonneuropathologic; x = neuropathologic.

in the spinal cord. In evaluating such an alteration the age of the individual must be considered.

4. ALTERATIONS IN THE MORPHOLOGY OF CENTRAL NERVE TERMINALS

It seems expedient at this point to insert observations on morphologic variabilities in the boutons in the neuropathologic cases. Preliminary observations of morphology in nonneuropathologic synapses have been reported [11] and it would seem permissible to assume that the conclusions for these cases apply, as well, to the group to be presented. These conclusions are: that variations in bouton "form" may be expected which are unrelated to the pathology, and that comparative data must be controlled as to region examined, nuclear column and age. Certain

conspicuous features (Hoff,[7,8] Gibson,[19] Marinesco,[20] Foerster, Gagel and Sheehan,[21] Schimert,[22] Snider [23]). Similar changes in neurofibrils of central nerve fibers (gn, Fig. 3) have been described in experimental poliomyelitis by Hurst.[24] Different degrees of bouton alteration are illustrated in Figures 1, 2 and 3. In Figure 1 all terminals are regular or thickened with the exception of the granular loop S″. In Figure 2 a relatively greater proportion of granular forms is in evidence and in Figure 3 nearly all the boutons appear granular. Figures 1 and 2 are from case H43, Figure 1 representing boutons around an intermediate sensory and internuncial cell, Figure 2 the terminals around an anterior horn cell. Figure 3 shows the terminals around the axon root on a cell of the nucleus dorsalis in case H7.

TABLE XIV

Group Data Showing Variations in Morphology and Number of Boutons, Arranged Categorically by Pathologic Processes

Myelitis or myelopathy	Cell sections	Total boutons	Per cent granular boutons	Represents increase over control average	Boutons per cell section
Arteriosclerotic (9 cases)	977	4598	21.84	3.31%	4.71
Metabolic, malnutrition, inanition (11 cases)	922	3889	29.83	11.30%	4.22
Toxic (11 cases)	1047	2301	31.46	12.93%	2.20
Neoplastic (10 cases)	1740	7214	31.67	13.14%	4.15
Traumatic (12 cases)	2382	10434	33.18	14.65%	4.38
Meningitis (11 cases)	1286	6410	35.32	16.79%	4.98
Other inflammatory (14 cases)	1469	6612	40.74	22.21%	4.50

5. THE INFLUENCE OF THE FUNDAMENTAL NATURE OF THE PATHOLOGIC PROCESS ON BOTH NUMBER AND MORPHOLOGY OF NERVE TERMINALS

The data of Table XIV indicate that quantitative differences in intensity or degree of alteration in both number and morphology of boutons may be related to the nature of the involvement. The values for the arteriosclerotic group are summarized from cases H77, H81, H55, H23, H18, H12, H6, H3, H31; those for the metabolic, malnutrition and inanition group, from cases H86, H11, H40, H44, H96, H88, H105, H104, H97, H9, H24; for the toxic group, from cases H56, H51, H47, H48, H60, H112, H73, H100, H103, H92, H82; for the neoplastic myelopathies, from cases H29, H37, H45, H17, H8, H49, H42, H50, H64, H62; for the traumatic group, from cases H1, H39, H16, H58, H74, H76, H75, H83, H91, H70, H99, H111; for meningitides,

Gibson [19] for criteria of alteration in the cat). In the comparisons to follow the thickened terminals will be included as regular forms.

Since age must be considered in relation to the proportion of granular boutons appearing in section, it is important to note the differences in Table XIII in which the cases are subdivided into age groups over

TABLE XII

Percentages of Regular, Thickened and Granular Boutons (Forms) in Nonneuropathologic and Neuropathologic Spinal Cords

	Regular	Thickened	Granular
Nonneuropathologic	79.64%	4.70%	15.66%
Neuropathologic	68.22%	4.27%	27.51%

and under 30 years. As might be expected from the previous report,[11] the difference between nonneuropathologic and neuropathologic cases is more striking in the younger group. The total average percentages for forms of terminals in the entire series of neuropathologic cases (except H113, H114, H115) were 31.41 per cent granular and 68.59 per cent regular boutons of 40,660 counted around 9700 cell sections. These

TABLE XIII

Percentages of Regular and Granular Boutons Appearing in Nonneuropathologic and Neuropathologic Cases in the Age Groups Under and Over 30 Years

	Regular boutons	Granular boutons
Under 30 years:		
Nonneuropathologic (5 cases)	92.01%	7.99%
Neuropathologic (9 cases)	75.15%	25.67%
Over 30 years:		
Nonneuropathologic (21 cases)	78.23%	22.20%
Neuropathologic (11 cases)	71.25%	28.75%

data indicated that, in general, granular forms of terminals appeared in greater proportion in cases with involvement of the central nervous system, and suggested, in view of the similar finding in aging, that the influential processes incident to both aging and direct pathology may be the same.

It should be noted here that the term, *granular,* is inadequate as a description of these altered terminals. Vesiculation, particularly of fibrillated bulbs, and swelling and paling of the neurofibrils are also

vidual notations in each category. It is evident that alteration in both number and morphology varied with the duration of the pathologic process. It is apparent, too, that change in number of boutons did not necessarily correspond in direction with morphologic change. Points that clarify these detailed discrepancies will be introduced in the discussions of individual cases.

7. THE INFLUENCE OF THE DISTANCE BETWEEN THE LESION AND THE ALTERATIONS OBSERVED

Referring to Table X it will be noted that endings adjacent to cells of the nucleus dorsalis appeared to be more numerous and those around somatic efferent groups less numerous in older individuals. Since

TABLE XV

Comparison Within Disease Categories, Illustrating the Influence of the Time Element on Synaptic Relationships

	Duration	Anterior horn cell sections	Boutons	Per cent regular	Per cent granular	Average no. boutons per cell section
Poliomyelitis (8 cases)	Less than 3 da. (3 cases)	155	1185	59.41	40.59	7.80
	More than 5 da. (5 cases)	291	1221	52.98	47.02	4.43
		Nucleus dorsalis cell sections				
Meningitis (11 cases)	Acute, less than 3 wks. (4 cases)	130	996	49.80	50.20	11.40
	Subacute, more than 4 wks. (4 cases)	93	228	52.83	47.17	2.88
	Chronic (3 cases)	45	341	65.85	34.15	7.71

Gardner [25] has shown that a diminution of myelinated dorsal root fibers may be expected after the third decade, the data mentioned above would appear to be erroneous unless it be assumed that the terminals of degenerating axons may persist. The boutons adjacent to the somatic efferent cells are probably, for the most part, terminals of short internuncial cells. The loss of the latter in the presence of persistent terminals of very long fibers suggests that related fiber length may have an important bearing on terminal change. This relationship is further suggested in the comparison of counts in acute and subacute cases of meningitis (Table XV). Where the irritative lesion was extramedul-

from cases H7, H53, H20, H27, H87, H102, H71, H98, H93, H85, H84; and for the other myelitides, from cases H5, H43, H41, H72, H109, H107, H89, H108, H110, H106, H94, H90, H101, H95. Inspection of Table I for the essential data of the cases in these groups will reveal a highly arbitrary selection. The placing of the three cases of old poliomyelitis in the group indicating traumatic effects requires the notation that the present lesion was considered a repair effect after loss of cells, the acute pathology having long since gone. Likewise, the diabetic cases in the metabolic group could undoubtedly be placed with the arteriosclerotic cases as well. With such limited data it was almost impossible to find exactly parallel cases adequate for comparison in a controlled series in order to detect effects due to differences in fundamental pathologic processes. Other than the general fact of variability in both number and morphology of terminals in the diseases arranged categorically, little may be said until the individual case data have been described. The striking diminution in the number of boutons per cell section in the toxic group was due to the fact that scarcely any boutons were demonstrable in the two cases of tetanus (H60, H112). The cases are regrouped according to synaptic pattern in section 9.

6. INFLUENCE OF THE DURATION OF THE PATHOLOGIC PROCESS ON THE NUMBER AND MORPHOLOGY OF TERMINALS

A suggestion that duration of the pathologic process may be importantly related to alterations in morphology of boutons is apparent in comparing the values for the acute diseases (particularly those inflammatory) with the values for chronic diseases (arteriosclerotic) in Table XIV. These two groups of cases may be thought of as similar with respect to general, widespread involvement but differ as to age and in the nature of the pathologic influence. The neoplastic and traumatic groups may not be included in such a comparison because of the specific location and extent of the lesions involved. More appropriate comparisons to illustrate the point are those in Table XV. The first compares the counts on anterior horn cells in 3 cases of poliomyelitis (H110, H90, H95), with a duration of 3 days or less, with counts on similar cells in 5 cases of the same disease (H101, H106, H94, H108, H41), with a duration of over 5 days. The second comparison illustrates the effect of duration of pathology on the cells of the nucleus dorsalis in 11 cases of meningitis subdivided into acute (cases H84, H85, H93, H7), subacute (cases H53, H102, H87, H98) and chronic (cases H20, H27, H71). In the subacute group all sections were histologically inflammatory, although the principal affection clinically was purulent meningitis. The values listed for boutons per cell section are averages of similar indi-

tion detectable at the synapse, but not completely as to the characteristics of the degenerative change. Phalen and Davenport [29] inclined to the belief that the variability in appearance at the synapse precludes the use of morphology as a criterion of degeneration. Barnard [9] failed to find progressive degenerative changes after appropriate experimental manipulation. He found, however, that boutons diminish in number following section of the axons of the cells about which they terminate (retrograde effect). This "axon reaction" is denied in the experimental work of Lawrentjew,[3] Barr [30] and Schadewald.[31]

The change in the terminals around cells of the nucleus dorsalis in the presence of an irritative lesion at the dorsal roots (Table XV, acute meningitis), and the diminution in number of terminals about cells of the dentate nucleus with loss of Purkinje cells (Table XVI) support the conclusion that alteration in boutons at the synapse is related to damage of the parent cell body or axon (progressive). By no means, however, is it implied that this alteration must, in every case, be considered degenerative. On the contrary, the alteration in boutons seems to be related frequently to increased synaptic activity, *e.g.*, increased number of terminals (beading effect) in early poliomyelitis (Table XV).

Data from case 53 in the previous report [11] indicated that axon reaction was absent around cells of the posterolateral group in the lumbar enlargement 5 months after amputation of the leg of the same side. Case H16, amputation of left leg 26 years previously, likewise showed no differences in counts of terminals around cells of appropriate columns of both sides in the lumbar enlargement. Further evidence that axon reaction appears to be absent in the human central nervous system is presented in Figures 4 and 5. The photomicrograph in Figure 4 shows persistent basket terminals at the sites of degenerated Purkinje cells in case H115 whose symptoms of Purkinje cell loss existed for 18 years (Table I). Figure 5 shows boutons adjacent to an anterior horn cell undergoing neuronophagia in a case of poliomyelitis (H106). Certain of these terminals are altered morphologically but it would seem this must be related to inflammatory effect on the cells or fibers supplying the afferent components of the synapse. To check this further, cells have been counted according to their appearance (uninvolved, damaged, or degenerated) at the time of examination in seven cases of poliomyelitis, and the nature of the terminals ending in relation to the cells noted (Table XVII). It is evident from the data that the alterations in the terminals are unrelated to the condition of the receptive component about which they end. Some terminals appeared to be engulfed by the neuronophagic cells, which may account

lary and confined to the entering fibers (acute cases) the terminals adjacent to the cells of the nucleus dorsalis were altered morphologically but did not seem to be diminished in number (actually increased); on the other hand, in the subacute cases (with intramedullary pathology) there appeared to be a diminution in number of terminals with approximately the same proportion of remaining terminals altered morphologically.

An opportunity to observe terminal effects from loss of cells with relatively short axons was afforded by the three cases of parenchymatous cortical cerebellar atrophy (H113, H114, H115). The pathologic manifestations in this disease include the disappearance of Purkinje cells from the cerebellar cortex with persistence of the basket terminals identifying the positions of degenerated cells (Parker and Kernohan[26]). In Table XVI data from these cases are compared with data from

TABLE XVI

Purkinje Cell Counts in the Cerebellar Cortex Correlated with Terminal Counts in the Dentate Nucleus of the Same Side

	Purkinje layer			Dentate nucleus			
	Normal cell sections	Degenerated cell sections	Per cent cells lost	Cell sections	Boutons	Boutons per cell section	Per cent boutons lost
Control (3 uninvolved)	150	0	0%	100	130	1.30	0%
Parenchymatous cortical cerebellar atrophy (3 cases)	41	261	86%	300	142	0.47	62%

uninvolved cases. It appears, assuming that all terminals ending around cells of the dentate nucleus are from Purkinje cells, that loss of Purkinje cells is reflected in the diminished number of boutons ending in relation to cells of the dentate nucleus. It is possible that all of the terminals of these cells do not disappear with degeneration of the cells (see Cajal,[2] for persistence of axon parts after section). Purkinje cell counts from flocculus and inferior vermis were excluded since the terminals of these cells probably end in relation to cells of the cerebellar roof nuclei.

8. PROGRESSIVE AND RETROGRADE EFFECTS AT THE SYNAPSE

That changes in boutons occur when their associated axons have been damaged experimentally is concluded from the work of Lawrentjew,[3] Gibson,[4] Hoff,[7,8] Foerster, Gagel and Sheehan,[21] Schimert,[22] Snider,[23] Hoff and Hoff[27] and Fulton, Hoff and Kennard.[28] These authors have agreed on the general principle of progressive degenera-